Immunohistochemistry and fluorescence in situ hybridization: practical aspects.

D’Haene Nicky

Sarah De Clercq

Pathological diagnosis: methodology

Macroscopy	Organ	TNM/ Staging
Microscopy	Tissue/cell	Classification/grading
Immunohistochemistry	Protein	Diagnostic Prognostic Theranostic
Molecular pathology	DNA, mRNA	Diagnostic Prognostic Theranostic

Biomarkers

Molecular Pathology

Tumor Biomarker

Tests should be performed on tumor cells

→ tests on the histological specimens

Optimization of molecular testing →

diagnostic

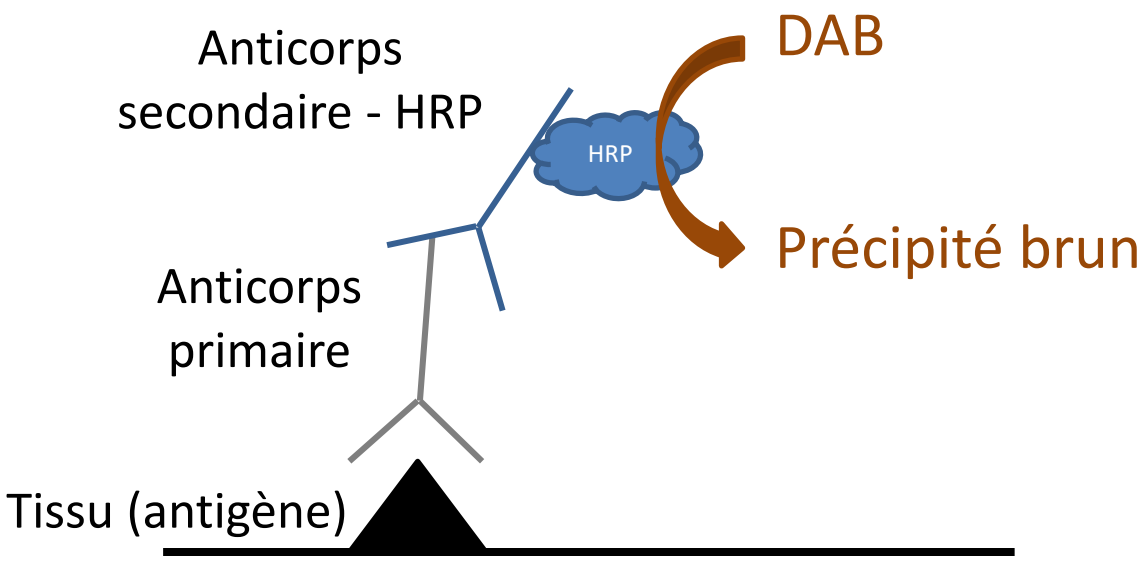
pronostic

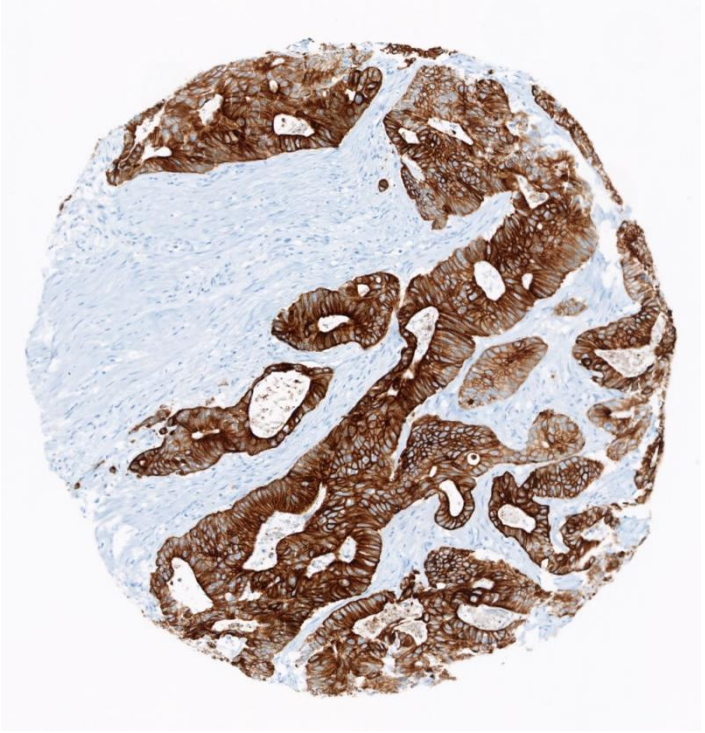
theranostic

Immunohistochemistry

Immunohistochimie

L'immunohistochimie (IHC) est une méthode qui permet de détecter des protéines dans des sections de tissu, basée sur les réactions antigènes-anticorps.





CK20 – ADC colon

Key elements in the immunohistochemical procedure

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Pre-analytic phase

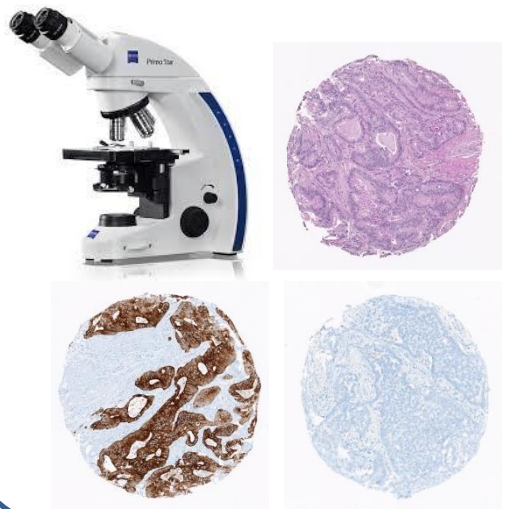
- ✓ Pre-fixation
- ✓ Fixative
- ✓ Fixation
- ✓ Post-fixation/decalcification
- ✓ Processing
- ✓ Dehydration & clearing
- ✓ Paraffin embedding
- ✓ Sectioning
- ✓ Drying/storage

Analytic phase

- ✓ Epitope retrieval
- ✓ Blocking
- ✓ Primary antibody
- ✓ Detection system
- ✓ Chromogen
- ✓ Counterstain
- ✓ Mounting
- ✓ Platform

Post-analytic phase

- ✓ Design of controls
- ✓ Internal/external controls
- ✓ Positive/negative controls
- ✓ Critical stain indicators
- ✓ Interpretation
- ✓ Quantification
- ✓ Cut-off level
- ✓ Reporting



Goldstein et al., Appl Immunohistochem Mol Morphol., 2007

The challenge of IHC

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Suboptimal IHC assays may be due to:

✓ **Pre-analytical issues:**

Choice of fixative, fixation too late, too short, too long, decalcification too soon, slide drying, slide ageing…

✓ **Analytical issues:**

Less successful antibodies/RTUs, inappropriate antibody dilution, inappropriate epitope retrieval, insensitive visualization systems, platform problems…

✓ **Post-analytical issues:**

Interpretation criteria, interobserver variation…

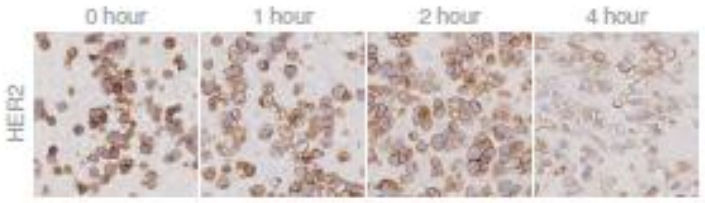
90 % of insufficient staining results in EQA are caused by weak/false negative results

→ **Necessity of biomarker controls**

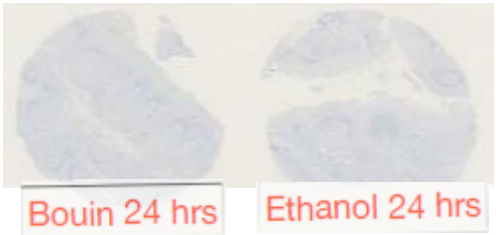
Mogens Vyberg, NordiQC

Pre-analytical issues

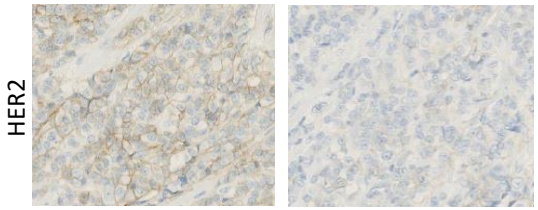
- Fixation delay:



- Type of fixative:
- Time of fixation:



- Slide drying:



1h à 60°C 16h à 80°C

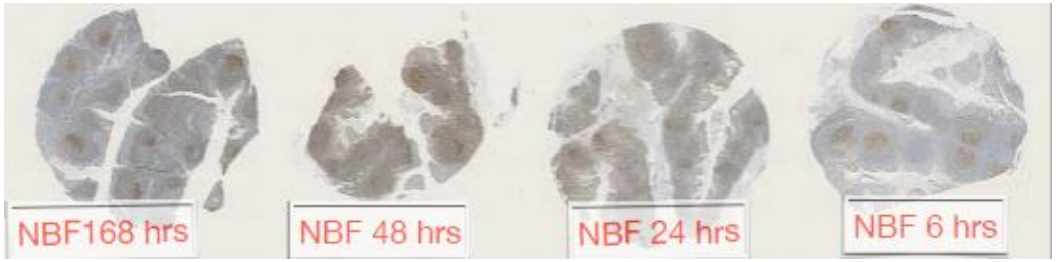


Table 4. Formal and Literature-Based Recommendations for Specimen Fixation and Processing Variables for Optimal Staining for Most Antigens Via Immunohistochemistry

Preanalytic Variable	Published Guidelines and Recommendations	Literature-Based Recommendations
Fixation delay	<1 h[8]	<12 h[1–4]
Fixation		
Fixative formula[a]	10% NBF, pH not specified[8,10]	10% NBF, pH 5–7[1,2,6,12]
Time in fixative[a]	24 h[8–10]	24 h[1,2,6,11,13,16–18,20–21]
Tissue to fixative ratio	1:10[8,10]	1:1 to 1:20[2]
Dehydration (total duration)	1.25–15 h[10]	10 h[1,2]
Paraffin impregnation		
Type of paraffin	Low-melting-point paraffin (55°C–58°C)[10]	Low-melting-point paraffin (45°C)[1]
Total duration	0.5–4.5 h[10]	1–2 h[1,28] or 8 h[2]
Slide drying	24 h at room temperature or 1 h at 50°C–60°C[10]	24 h at room temperature[1] or overnight at 37°C[2]
Storage duration		
Paraffin block[a]	Indefinitely[10]	≤25 y[16,33–35]
Slide-mounted sections[a]	7 d[9] or <6 wk[8]	<6 d[38]

4°C better than RT

6 – 48h

Abbreviation: NBF, neutral-buffered formalin, 10% = 4% neutral buffered formaldehyde

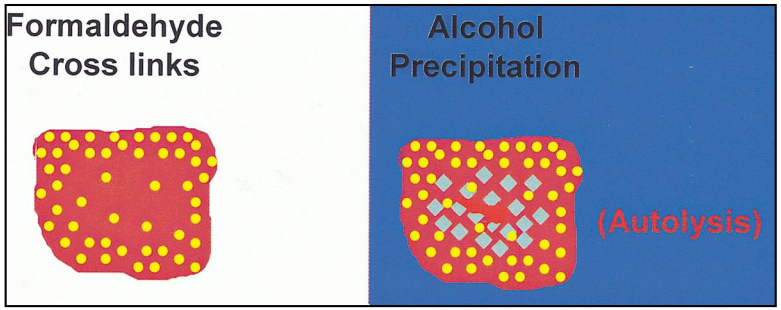
Engel et al., Arch. Pathol. Lab. Med., 2011

Fixation

Fixation courte

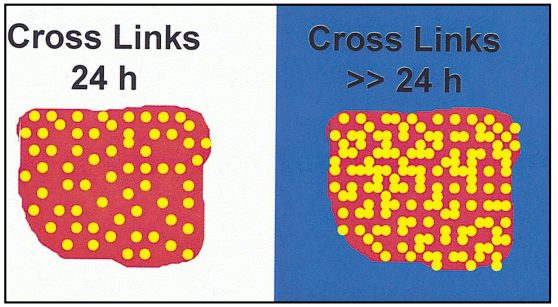
– Fixation mixte

– Additive + coagulative



Fixation longue

- Altération ou destruction des épitopes
  - Susceptibilité individuelle
- Formation excessive des liens
- Contamination par des impuretés



Fixation optimale : fragments de 5 à 10 mm fixés dans 20 x le volume tissulaire pendant 24 à 48 h

Werner et al, Am J Surg Pathol, 2000

Fixation

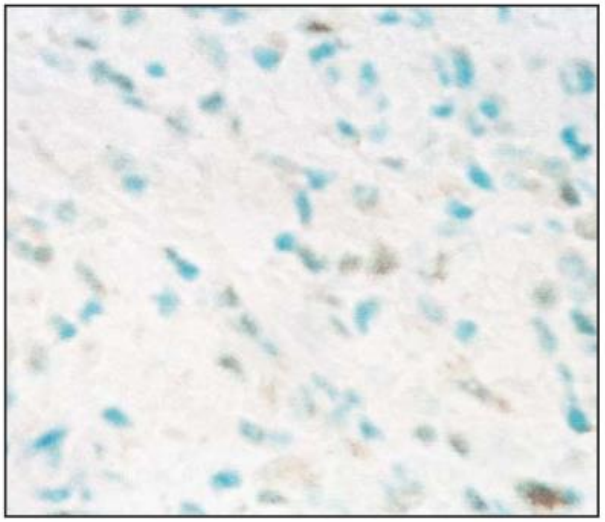


Image 1 Fixation, 3 h; antigen retrieval, 40 min.

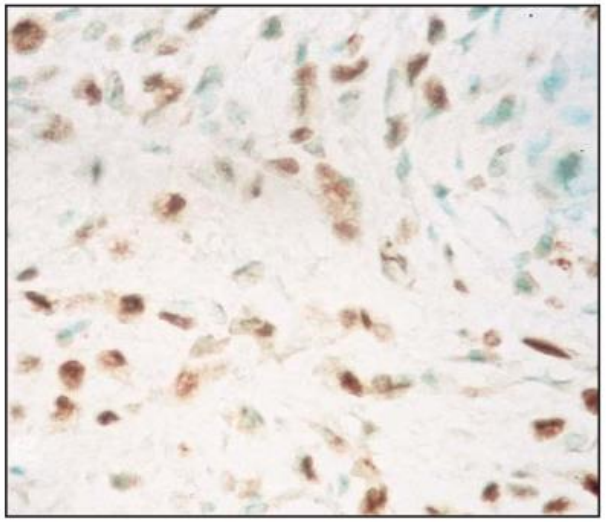


Image 2 Fixation, 6 h; antigen retrieval, 40 min.

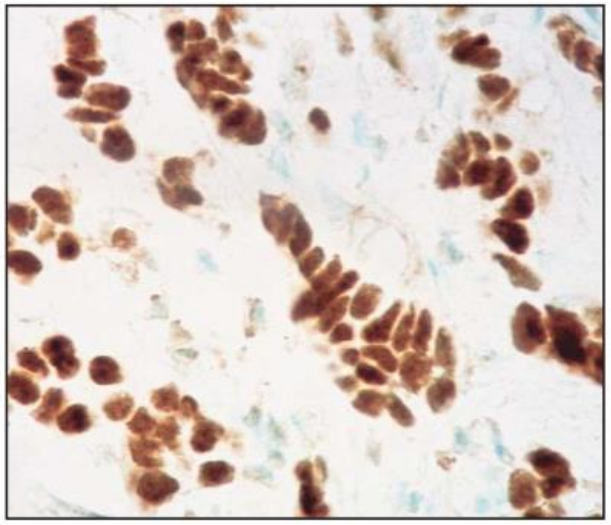


Image 3 Fixation, 8 h; antigen retrieval, 40 min.

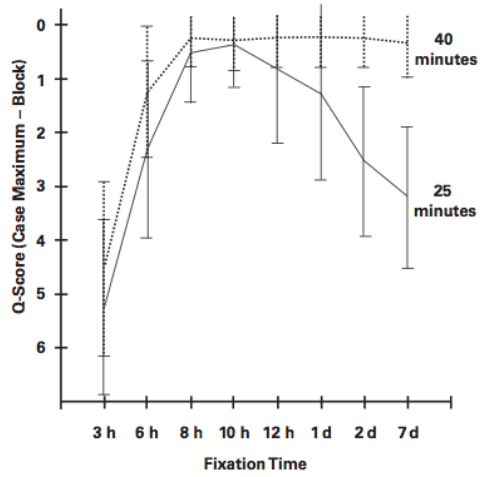


Figure 1 Plateau, case maximum (max) estrogen receptor (ER) scores occurred at 6-8 hours. Standard immunohistochemical method used 40 minutes of antigen retrieval.

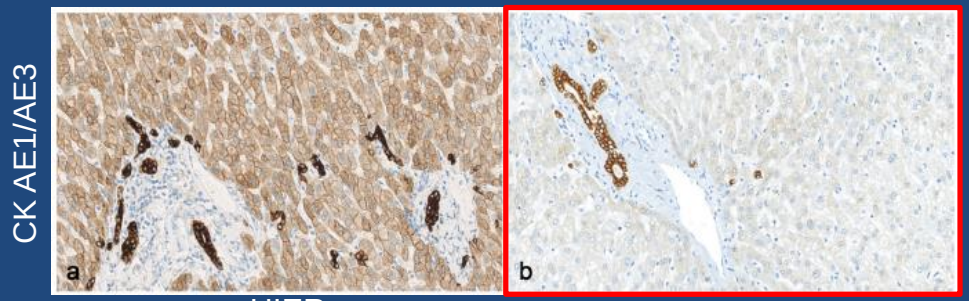
Goldstein et al, Am J Clin Pathol, 2003

Analytical issues: pre-treatment

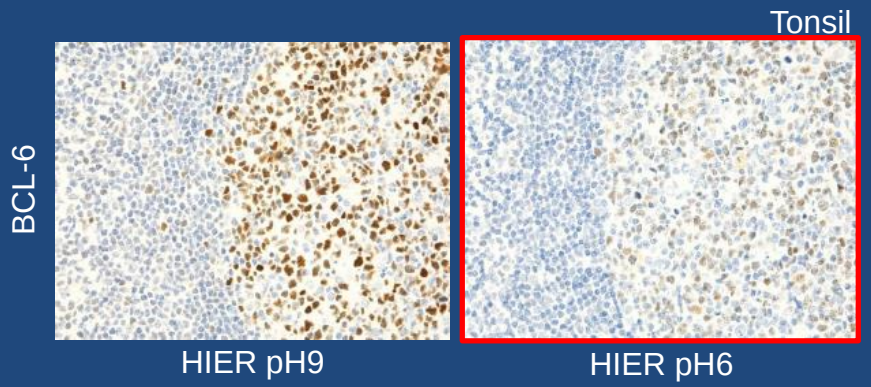
Table 3 Major causes of insufficient staining reactions

1. Less successful antibodies (17 %)
   a. Poor antibodies[a]
   b. Less robust antibodies[b]
   c. Poorly calibrated RTUs
   d. Stainer platform dependent antibodies
2. Insufficiently calibrated antibody dilutions (20 %)
3. Insufficient or erroneous epitope retrieval (27 %)
4. Error-prone or less sensitive visualization systems[c] (19 %)
5. Other (17 %)
   a. Heat-induced impaired morphology
   b. Proteolysis induced impaired morphology
   c. Drying out phenomena
   d. Stainer platform-dependant protocol issues
   e. Excessive counterstaining impairing interpretation

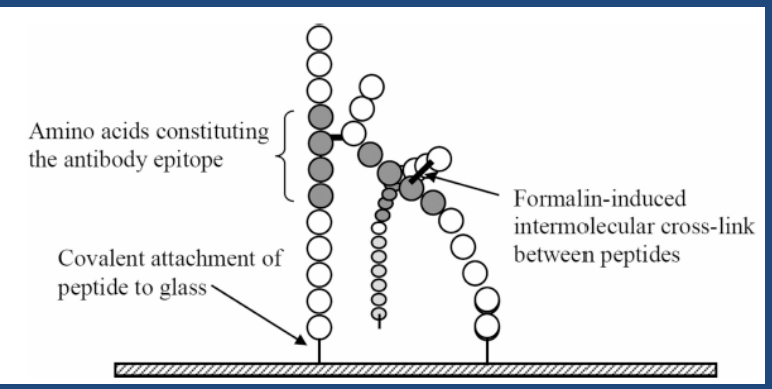
Liver

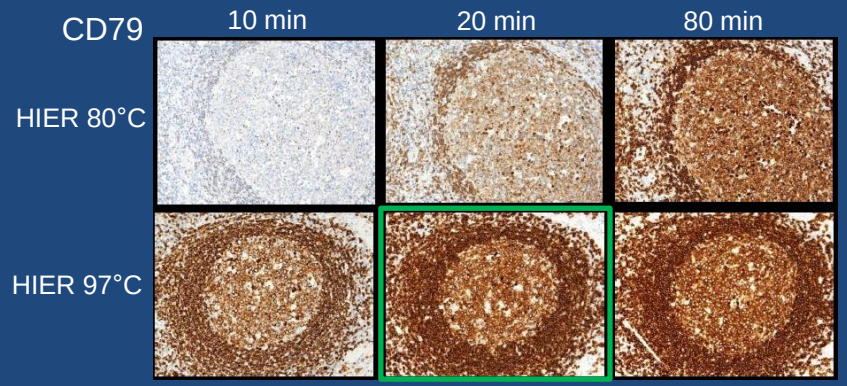


Tonsil



Effect of formalin fixation on peptide epitopes:





Michael Bzorek, NordiQC

Bogen at al., Biotech. Histochem., 2009

Vyberg and Nielsen, Virchows Arch., 2016

Analytical issues: primary antibody

Table 3 Major causes of insufficient staining reactions

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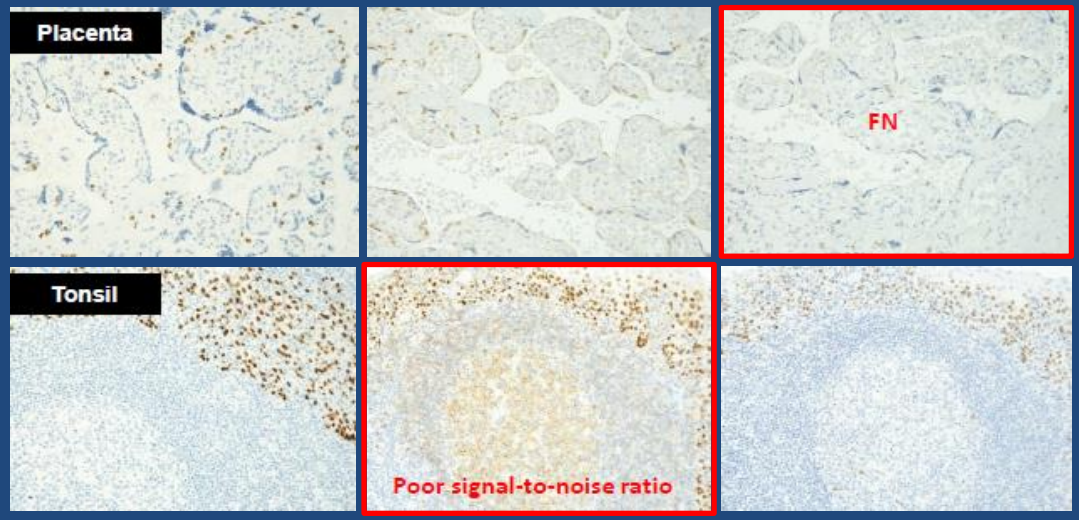
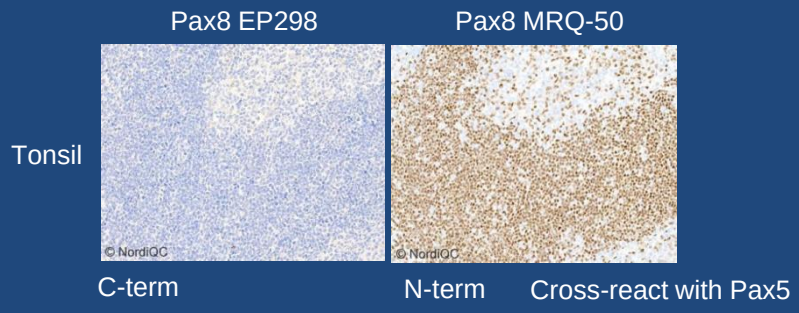
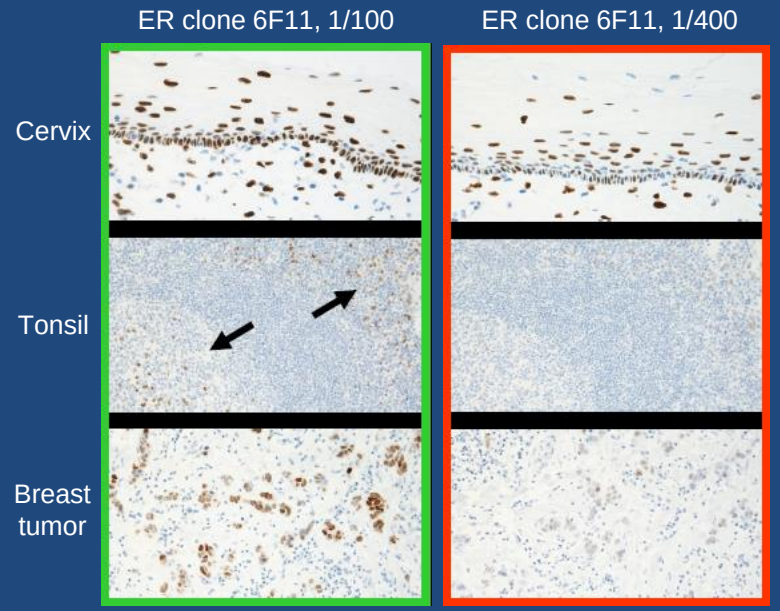




Low specificity



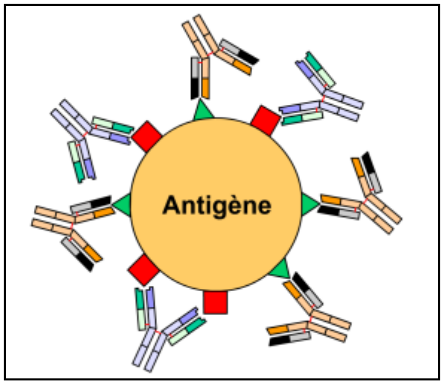
Low sensitivity

Michael Bzorek, NordiQC

Anticorps

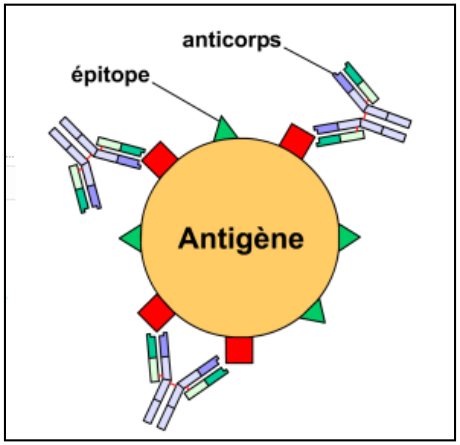
Polyclonal

- Liaison à des épitopes différents
- Mélange d'Ac
  - Ig classe et sous-classe ≠
  - Chaînes légères κ et λ
  - Différents paratopes liant les différents épitopes
  - Affinités ≠



Monoclonal

- Liaison spécifique à un épitope
- Un seul type d'Ac
  - Une seule classe
  - Un seul isotype de chaîne légère : κ et λ
  - Liaison à seul épitope
  - Affinité unique



Anticorps

Polyclonal

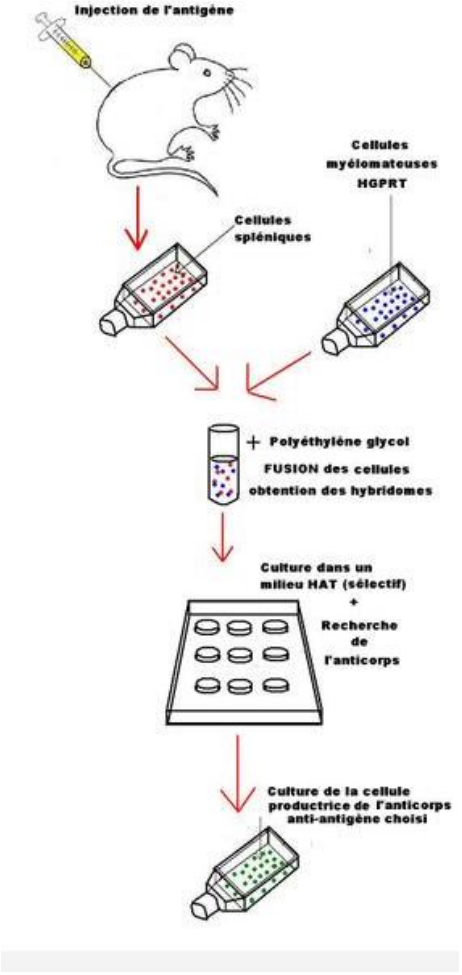
Injection de l'Ag

- Porte plusieurs épitopes
- Plusieurs lymphocyte B vont être activés et se diviser
- Production de plusieurs clones

Récupération du sérum

- Ac dirigés contre la protéine d'intérêt mais aussi d'autres protéines
- Ac de classes et sous-classes ≠

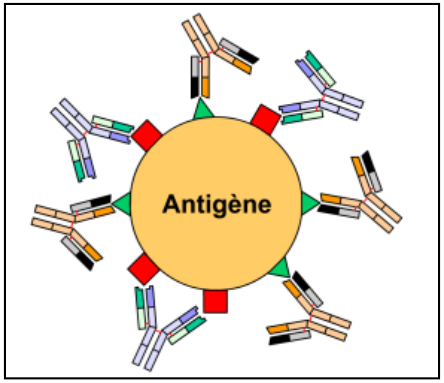
Monoclonal



Anticorps

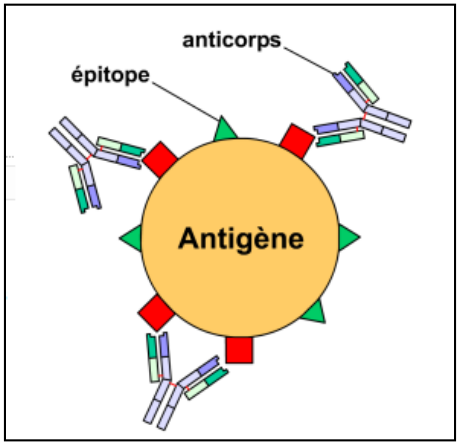
Polyclonal

- Avantage
  - Sensibilité élevée
- Inconvénients
  - Manque de spécificité
  - Manque de reproductibilité
  - Quantité limitée
  - Variation d'un lot à l'autre



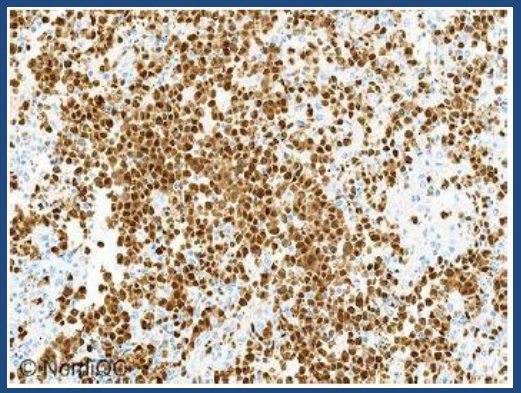
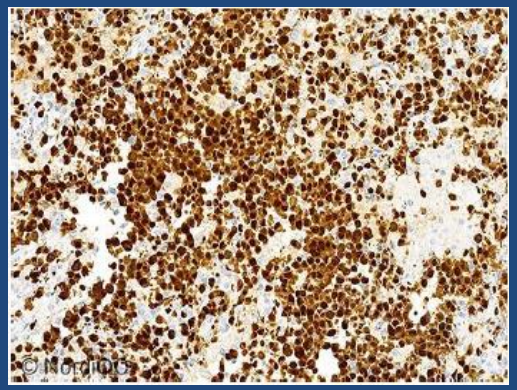
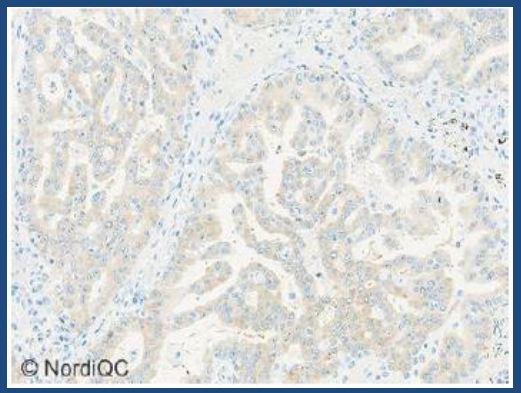
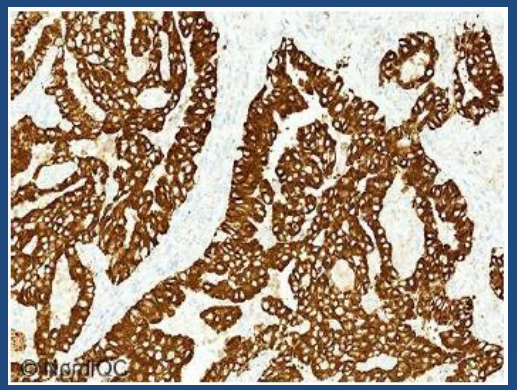
Monoclonal

- Avantages
  - Spécificité élevée
  - Immortalité de l'hybridome
- Inconvénient
  - Sensibilité faible



«Fit-for-purpose» assay

What ultimately makes a laboratory test « bad » or « good » depend on wether or not the test is properly validated (clinically, diagnostically and technically) for the specified purpose (« fit-for-purpose »).

ALK	ALK1	D5F3
ALCL		
NSCLC		

Different gene rearrangements and different protein expression levels

Higher sensitivity required

An assay that is “fit-for-purpose” is good enough to do the job it was designed to do

Michael Bzorek, NordiQC

Cheung et al., Appl Immunohistochem Mol Morphol; 2017

Diagnostic specificity vs sensitive antibody

Higher sensitivity

Clone SPT24

TTF1

Clone 8G7G3/1

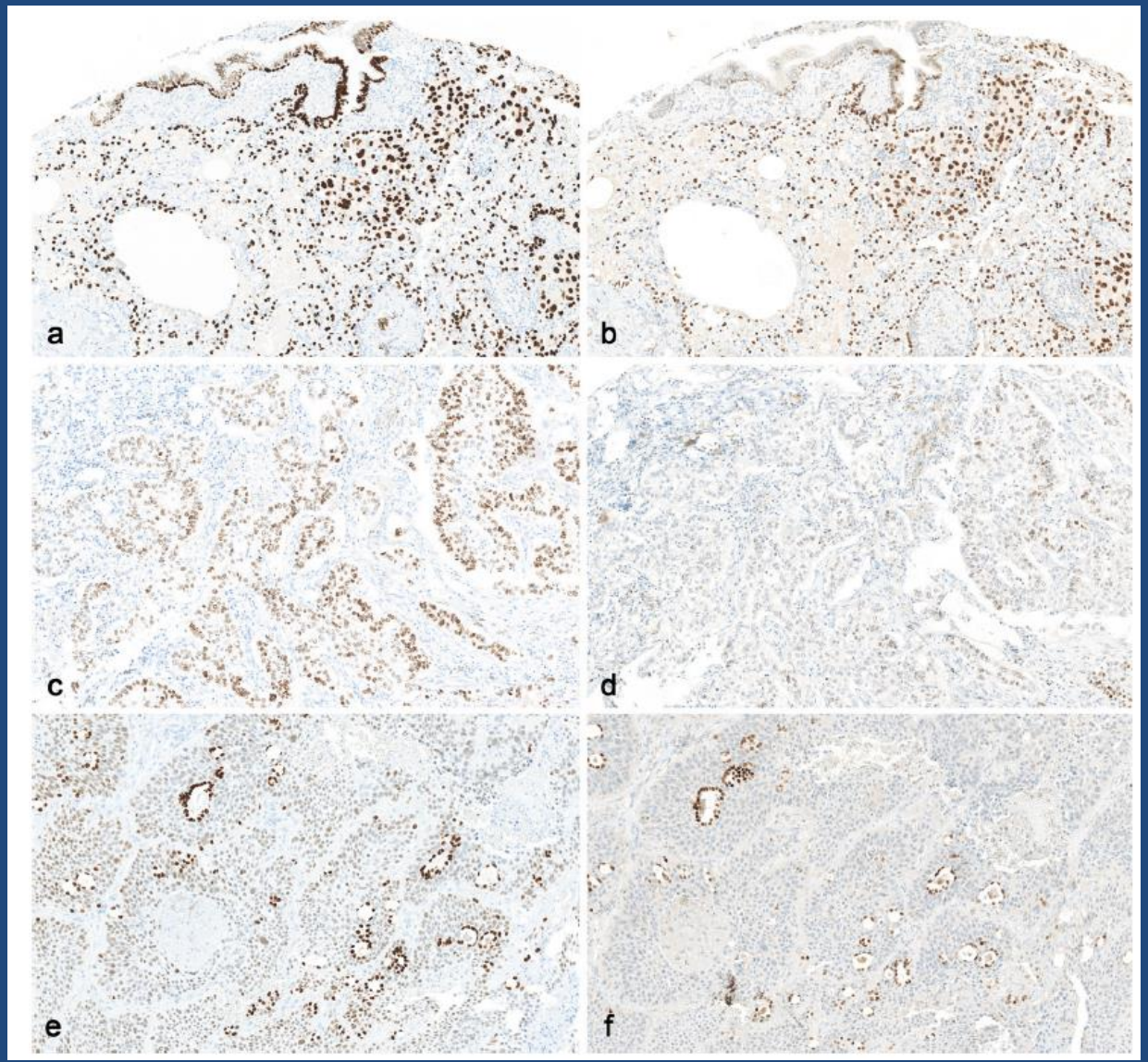
Higher specificity

WHO recommended

ADC

+ in 72 - 84%

+ in 65 - 77%



SCC

+ in 17%

+ in 1%

Kadota et al., Am J Surg Pathol, 2015
Vyberg and Nielsen, Virchows Arch, 2016

Analytical issues: detection system

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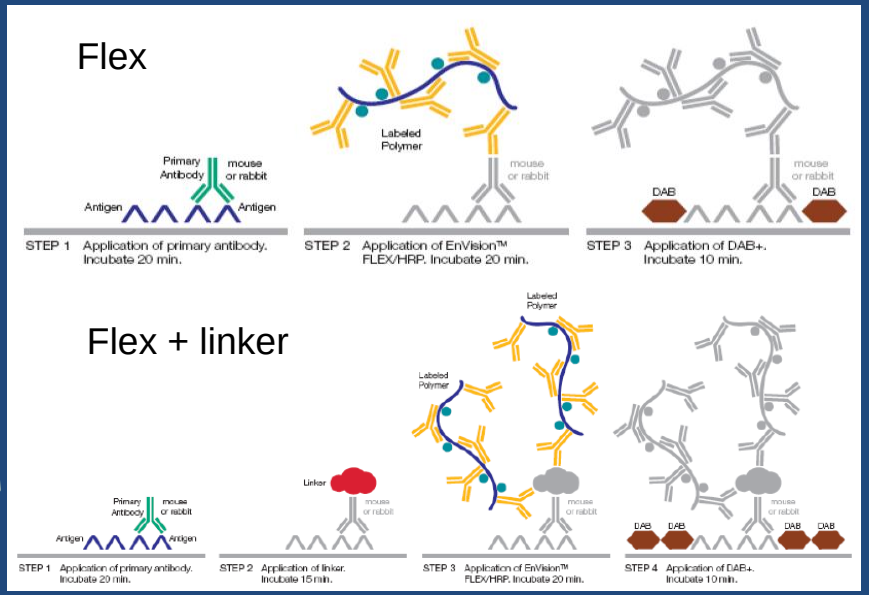
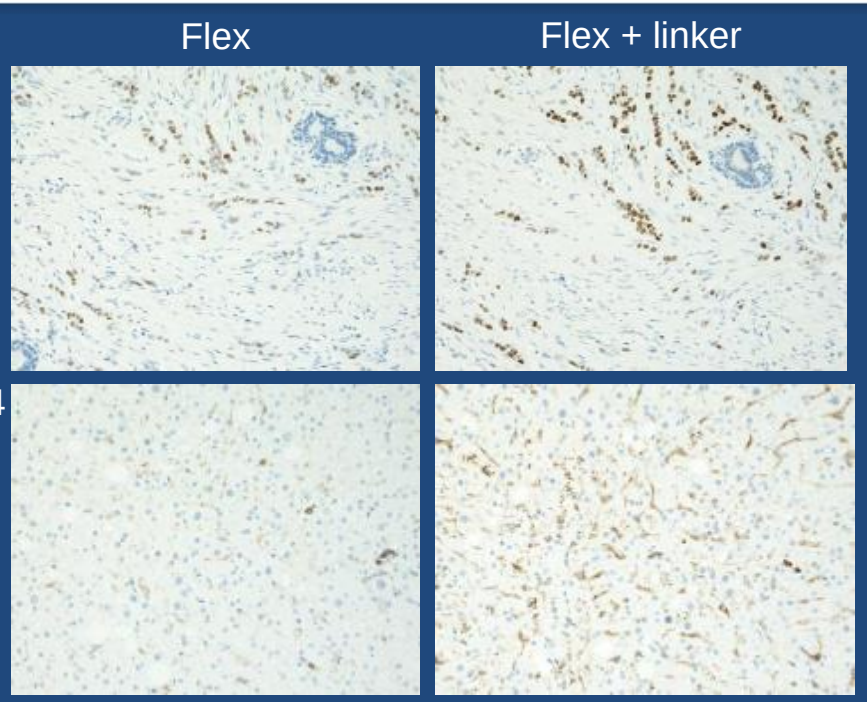
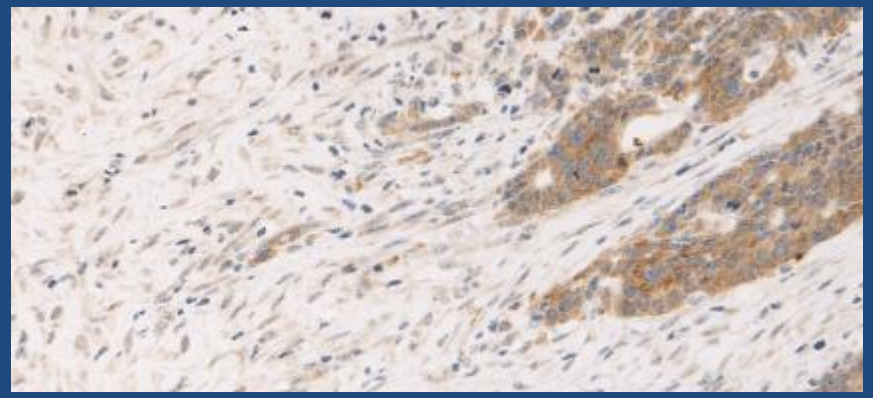


sensitivity



Flex + linker



Background!

Michael Bzorek, NordiQC

Post-analytic: Biomarker controls

Controls are essential to evaluate IHC results:

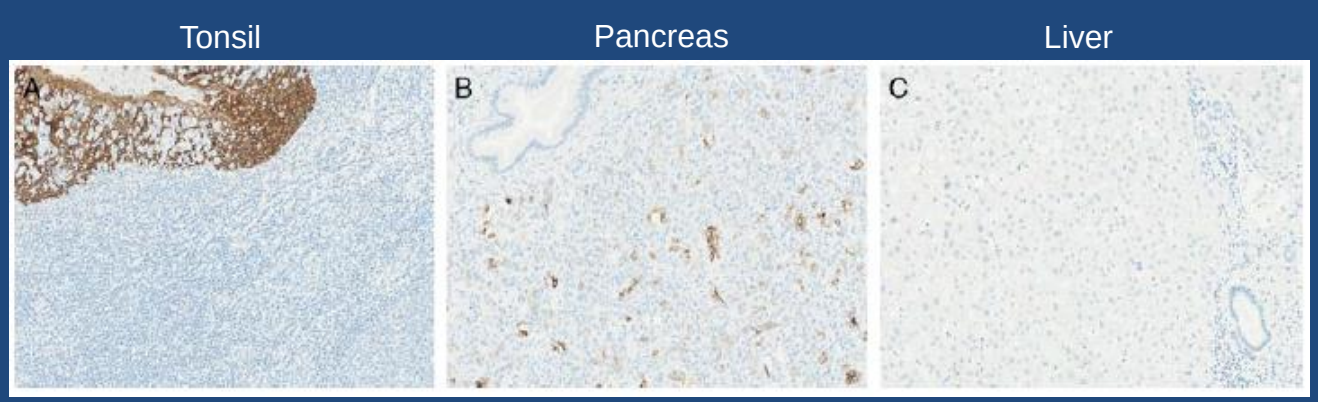
1) Tissue controls used to calibrate IHC assay (identification of best practice protocol)

2) Tissue controls processed by variables applied in the laboratory is needed to evaluate on robustness (pre-analytic controls)

3) Tissue controls to evaluate analytical potential (sensitivity – specificity)

4) Tissue controls to monitor consistency of IHC assay (daily practice controls)

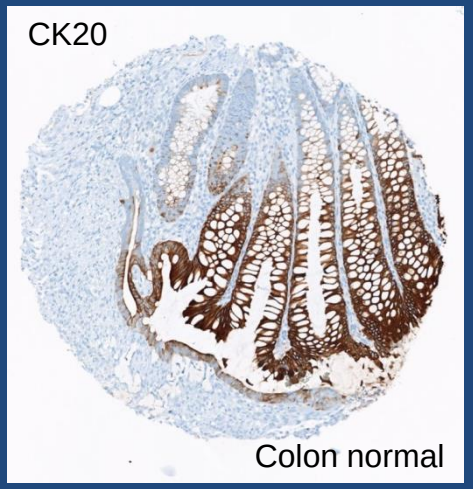
AIM: To confirm that the IHC result can be trusted and subsequently used to analyze our specimen.



Tissue with **high epitope expression** to identify the right antibody

Tissue with **low epitope expression** to assure the sensitivity

Tissue with **no epitope expression** to assure the specificity



Post-analytique: critères d'interprétation

Critères d'interprétation

→ type de marquage attendu ? (lecture critique)

→ score ? En fonction de la pathologie?
Ex : PD-L1 – poumon : TPS / PD-L1 – ORL et URO: CPS
HER2 gastrique/sein

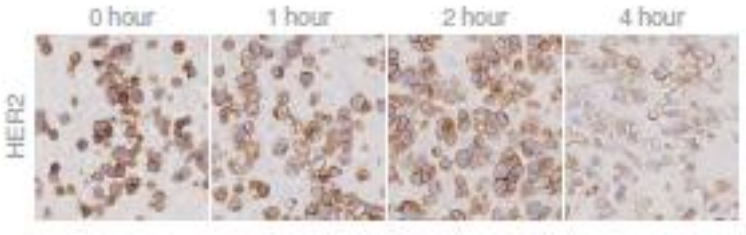
Seuils différents (implication thérapeutique ++)

→ formation du pathologiste

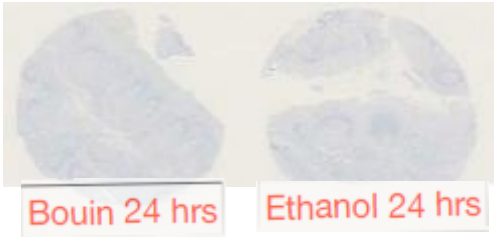
→ quantification?
→ IA

Tumor Indication	PD-L1 Expression Level
NSCLC	TPS ≥ 1%
Urothelial Carcinoma	CPS ≥ 10
HNSCC	CPS ≥ 1

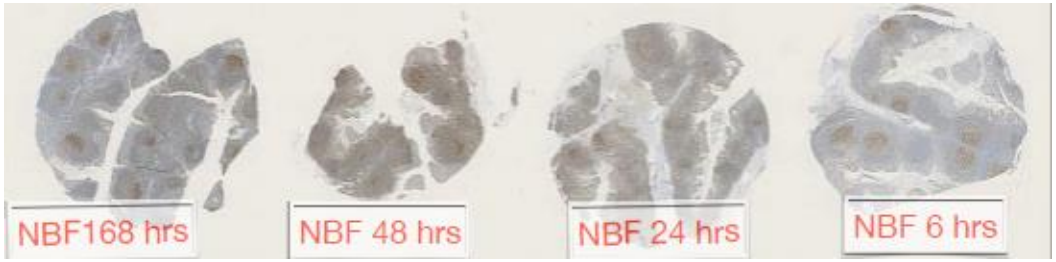
Résumé : Immunohistochimie: étapes pré-analytiques



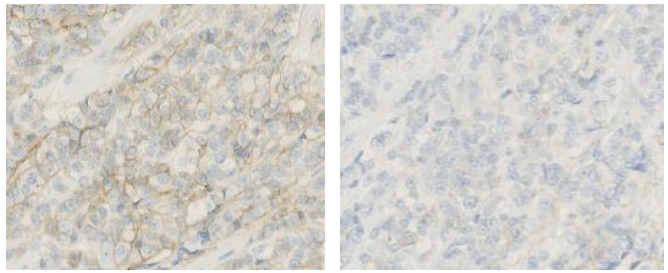
- PRELEVEMENT
  - → Délais avant fixation (temps d'ischémie) : < 1h
- FIXATION
  - → Taille du prélèvement : Ratio tissu – fixateur: 1:10
  - → Type de fixateur : 4% NBF
  - → Temps de fixation : 24h (8-72h)



PMS2



- INCLUSION/ENROBAGE
- COUPE
  - → Epaisseur : 4 µm
  - → Séchage : 24h à RT, 1h à 60°C, O/N à 37°C

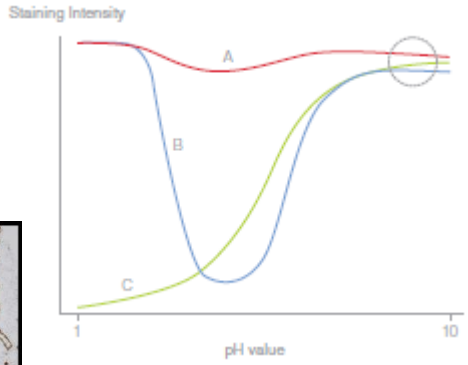


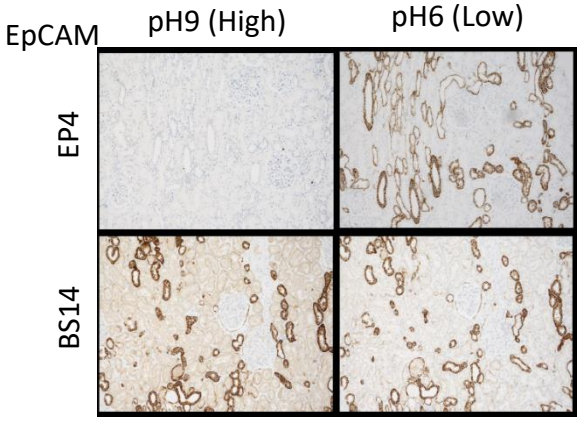
1h à 60°C

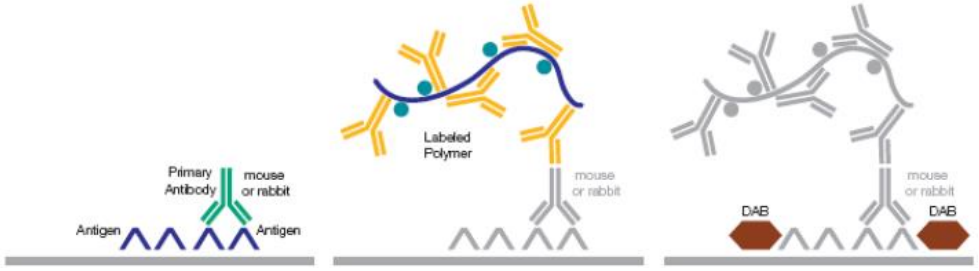
16h à 80°C

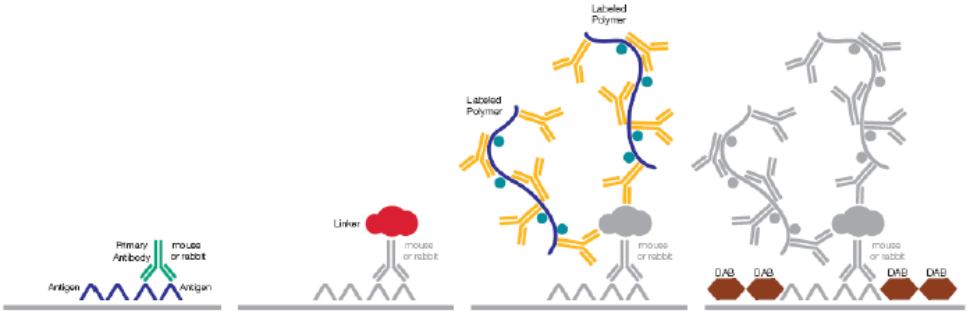
Résumé : Immunohistochimie: étapes analytiques

- DEPARAFFINAGE
- PRE-TRAITEMENT (démasquage des antigènes)
  - → Digestion enzymatique
  - → Chaleur
  - → Temps
  - → Tampon Citrate pH6 – EDTA pH9
- ANTICORPS PRIMAIRE
  - → Monoclonal (clone), polyclonal
  - → Spécificité, sensibilité
  - → Concentré (dilution, tampon), RTU
  - → Temps, température
- BLOCAGE PEROXYDASES ENDOGENES
- SYSTÈME DE VISUALISATION
  - → Avec ou sans amplification
- CHROMOGENE
  - → HRP-DAB, AP-RED
- CONTRE-COLORATION





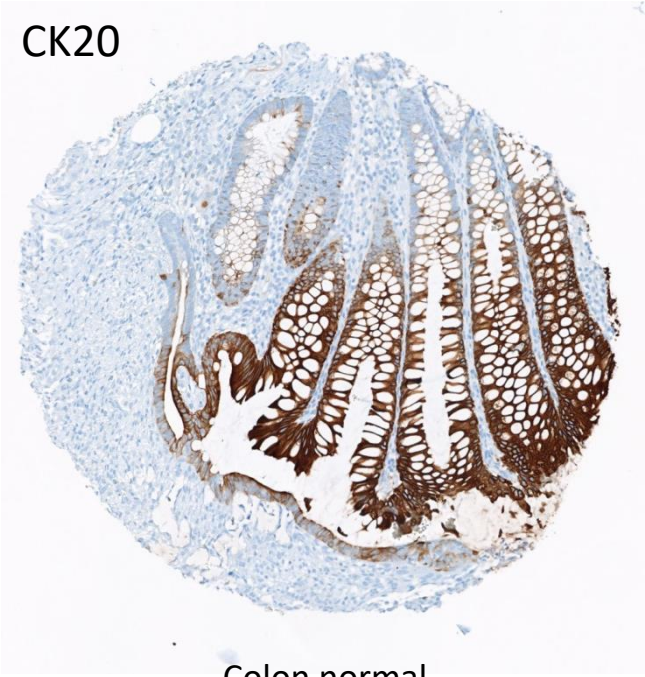




Résumé : Immunohistochimie: étapes post-analytiques

- CONTROLES
  - → Un tissu contrôle associé à chaque anticorps
  - → Négatif, Positif et faible expression
  - → Contrôle interne

- INTERPRETATION
  - → Connaissance de la localisation antigénique précise (nucléaire, membranaire, cytoplasmique…)
  - → Types de marquage (diffus, focal, granulaire)
  - → Quantification
  - → Marquage positif → seuil (proportion de cellules +)
  - → Hétérogénéité, variation d'intensité

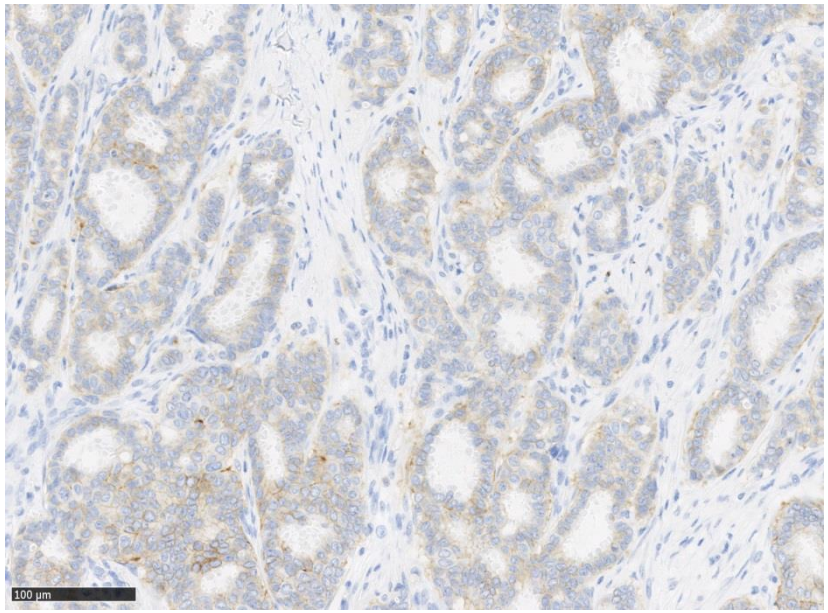


Colon normal

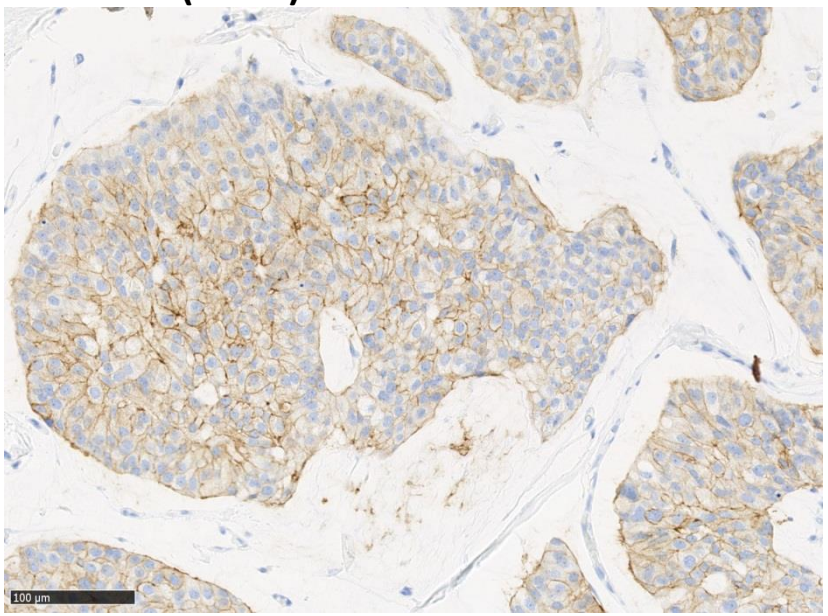
Source: fiche technique de l'anticorps, NordiQC, The Human Protein Atlas…

HER2 IHC

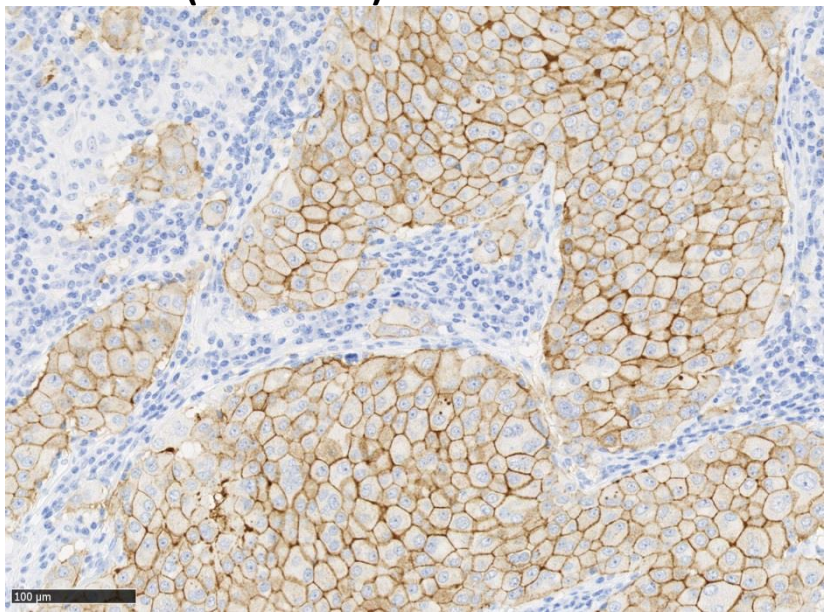
Score 0/1+



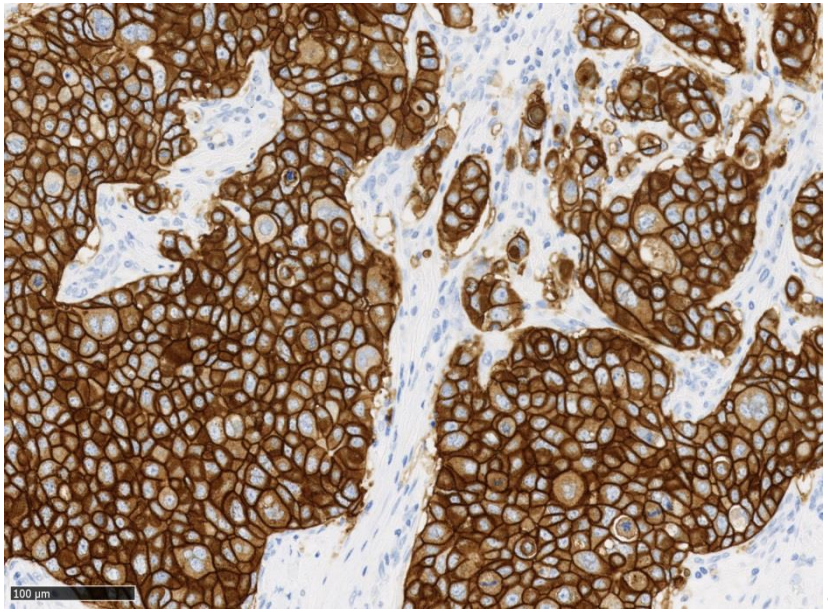
Score 2+ (weak)



Score 2+ (moderate)



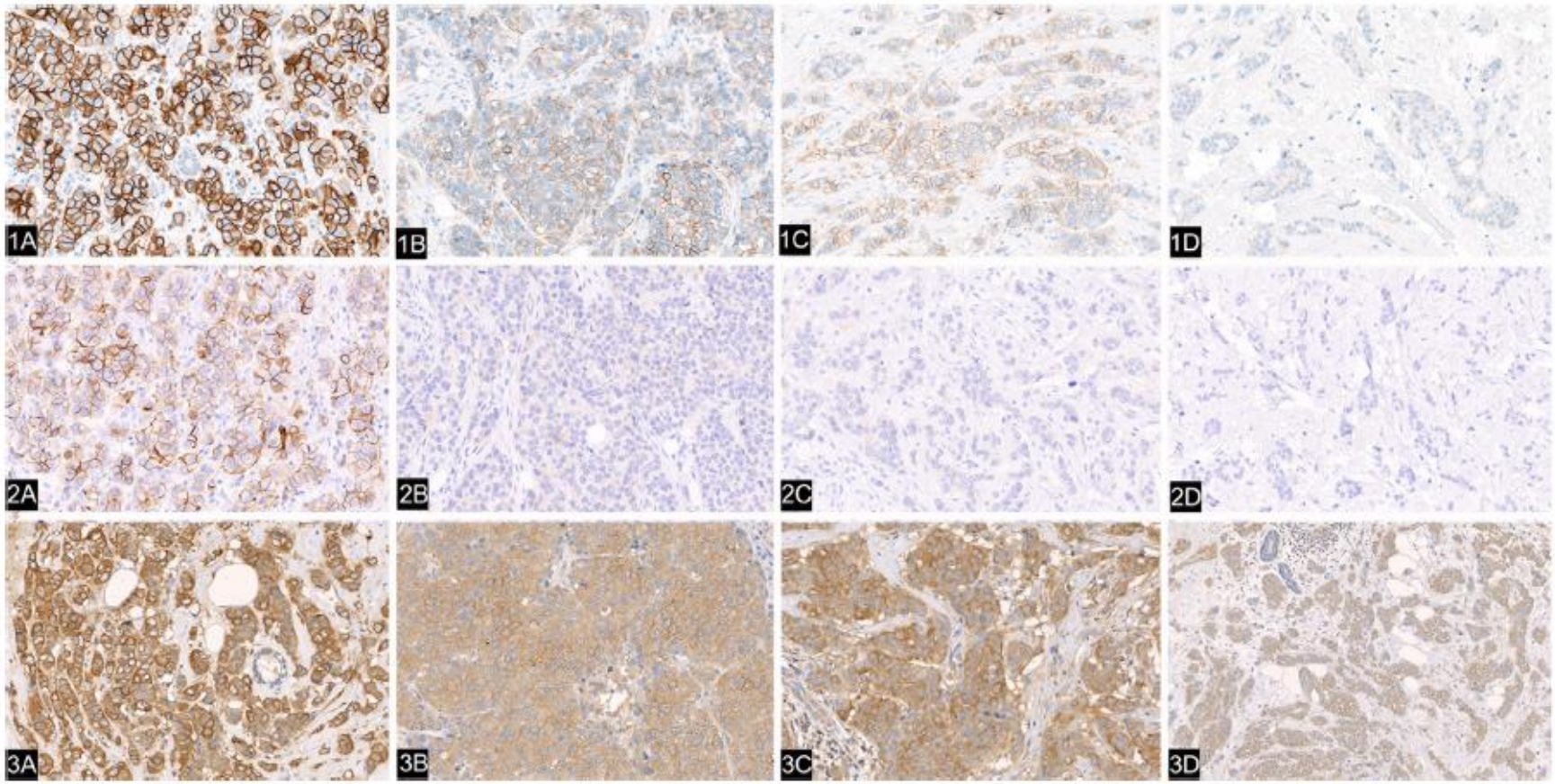
Score 3+



20X

Scoring Variability factors

- Accurate and inaccurate staining (optimal and insufficient protocols)



✓

FN

X

FP

X

Table 1 FP and FN rates for immunohistochemical testing as recorded by the NordiQC programme

	Approved IVD, n (%) (n = 1145)		Laboratory-developed IVD, n (%) (n = 558)	
Data source	FN	FP	FN	FP
NordiQC runs B6–14[a]	127 (11)	0	141 (25)	28 (5)

FN false negative, *FP* false positive, *IVD in vitro* diagnostic, *NordiQC* Nordic Immunohistochemistry Quality Control Group
[a]NordiQC IHC quality-control organisation, B6–14 runs [15]. Laboratories were provided with samples that were IHC 0, 1+, 2+ and 3+

Vyberg et al. BMC Health Services Research (2015)

Scoring Variability factors

- Intratumoral HER2 heterogeneity

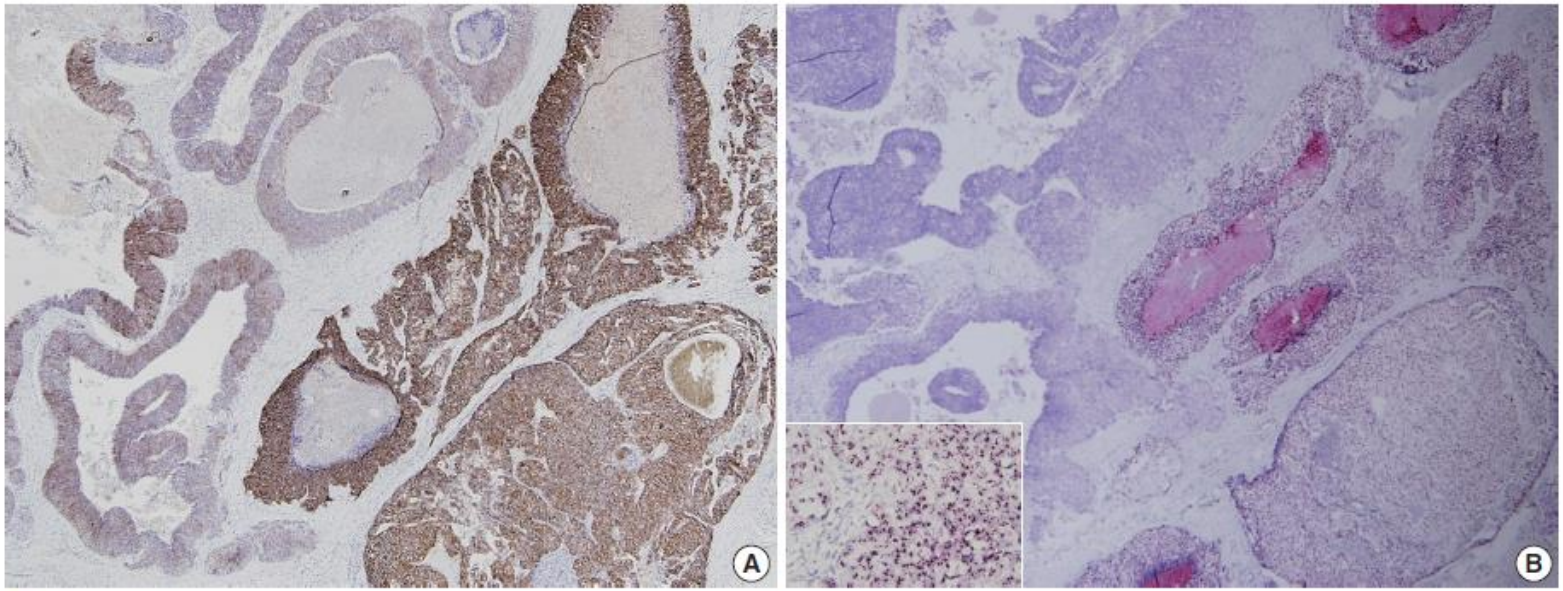
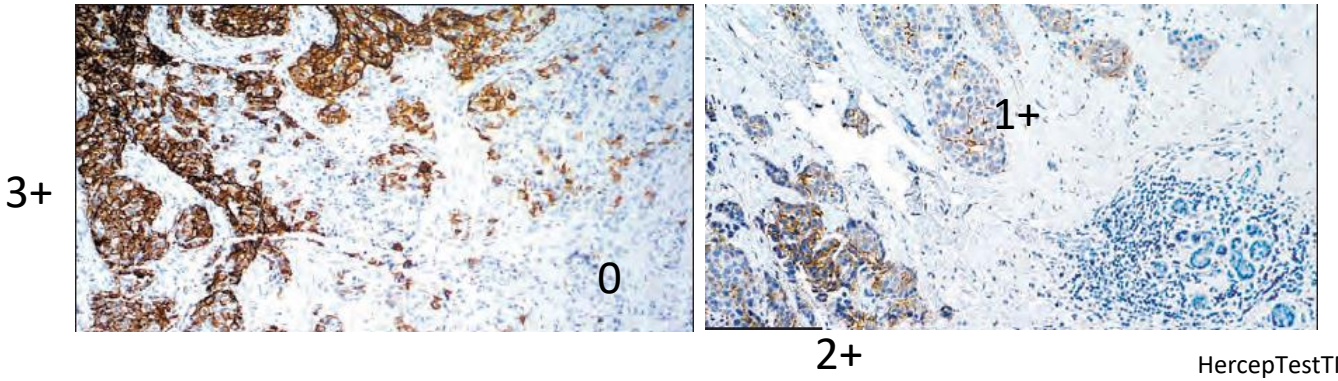


Fig. 2. A representative breast cancer with intratumoral human epidermal growth factor receptor 2 (HER2) heterogeneity. (A) HER2 immunohistochemistry shows heterogeneous expression with strong, complete membranous expression on the right, and weak to moderate, incomplete membranous expression on the left. (B) HER2 silver in situ hybridization reveals high-level amplification on the right and no amplification on the left (inset, area of high-level amplification).

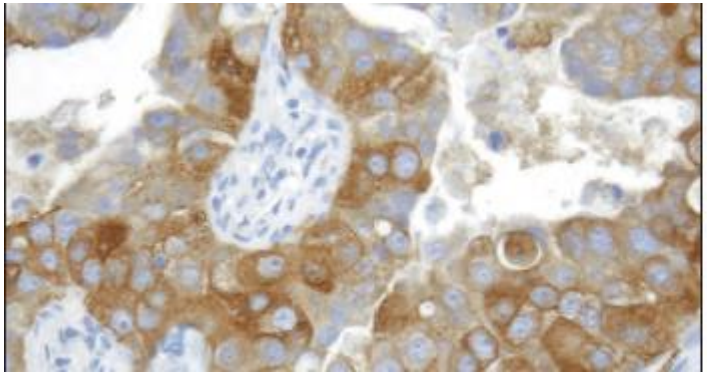
Ahn S et al. Journal of Pathology and Translational Medicine 2020

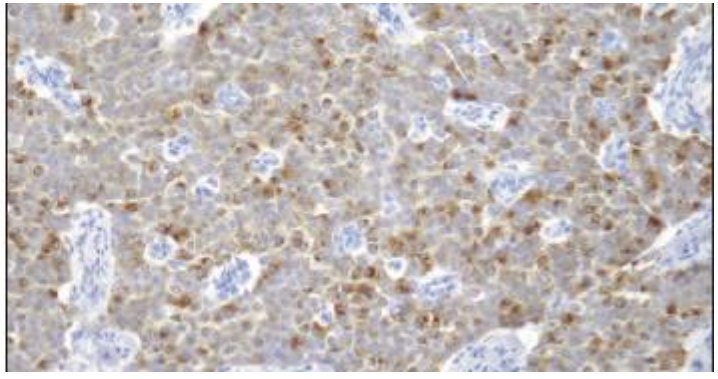


HercepTestTM Interpretation Manual Breast Cancer

Scoring Variability factors

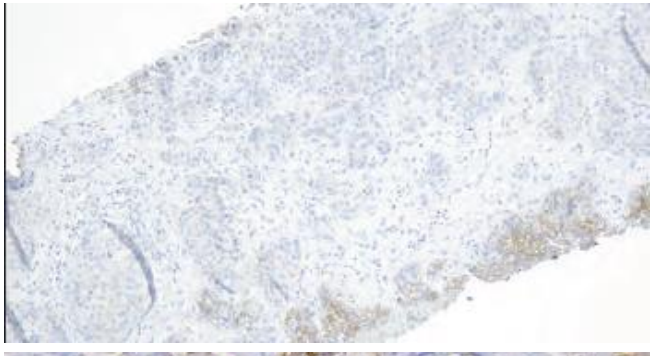
- Cytoplasmic staining



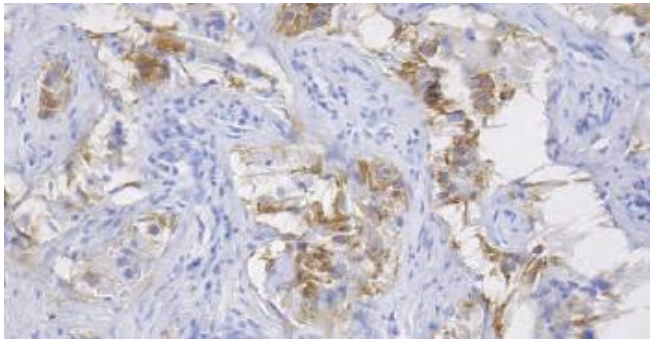


Score 0

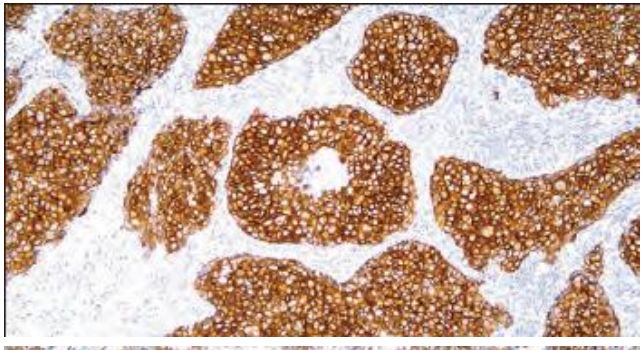
- Artifacts: fixation, temperature



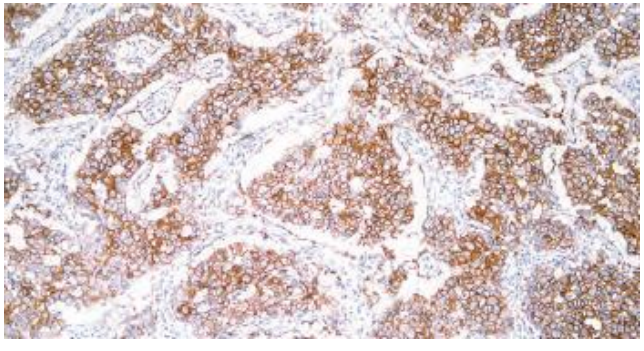
« Edge »



Retraction



Same tumor

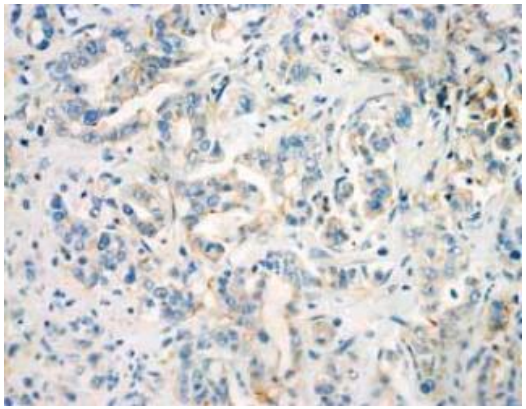


Too long fixation

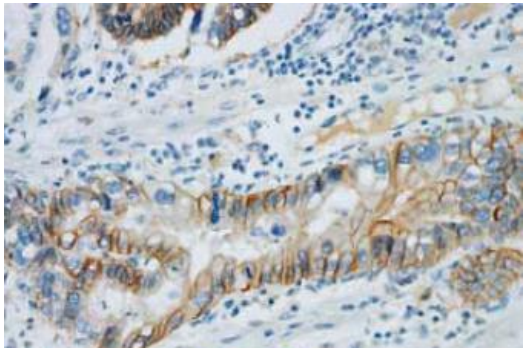
HER2 gastric cancer

Table 1. Algorithm for reproducible intensity scoring in HER2 IHC in gastric cancer (magnification rule).

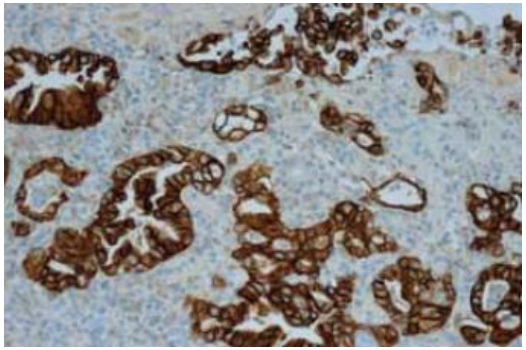
Score 3+: Tumour cell clones with a strong basolateral or lateral membranous reactivity irrespective of percentage of tumour stained	Positivity directly visible by eye or at low magnification (5X)
Score 2+: Tumour cell clones with a weak to moderate basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Requires a more detailed magnification with a 10X or 20X objective for demonstration of membranous staining
Score 1+: Tumour cell clones with a faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	If high magnification, a 40X objective, is required for demonstration of membranous staining



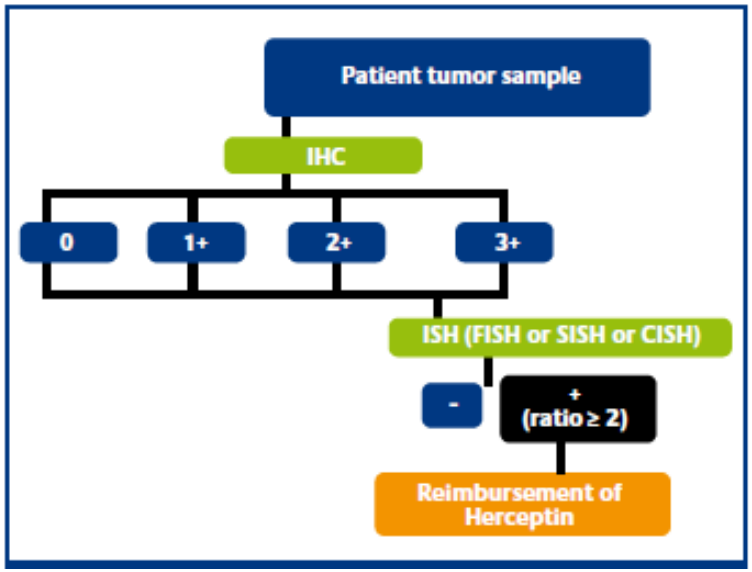
1+



2+



3+



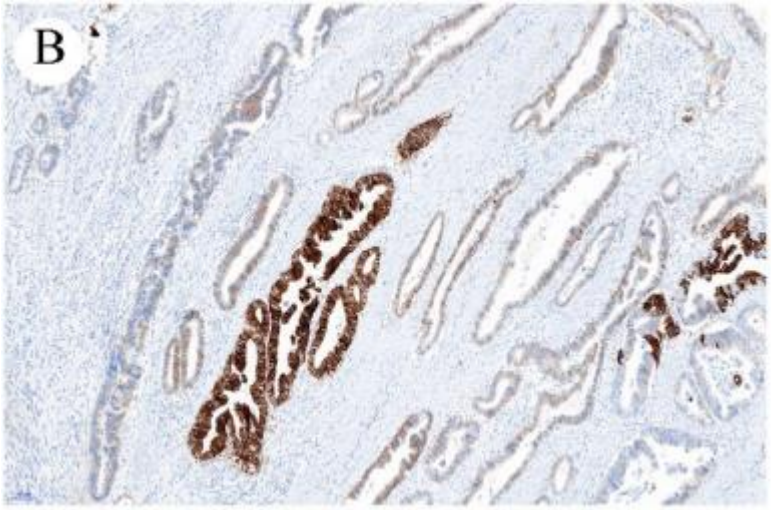
Belgian guidelines for HER2 testing in gastric cancer, Belg J Med Oncol 5:14-22, 2011

HER2 gastric cancer

Table 1 Comparison of expression of HER2 and amplification of *HER2* gene in gastric carcinomas

Antibodies	*HER2* FISH	
	Amplified ($n=38$)	Not amplified ($n=251$)
HercepTest		
Positive (3+)	22	0
Positive (2+)	8	10
Negative (0/1+)	8	241
A0485		
Positive (3+)	23	1
Positive (2+)	9	13
Negative (0/1+)	6	237
4B5		
Positive (3+)	24	2
Positive (2+)	5	9
Negative (0/1+)	9	240
CB11		
Positive (3+)	21	0
Positive (2+)	2	4
Negative (0/1+)	15	247

Focal HER2 gene amplification is more common in GCs with no (0 and 1+) HER2 expression.



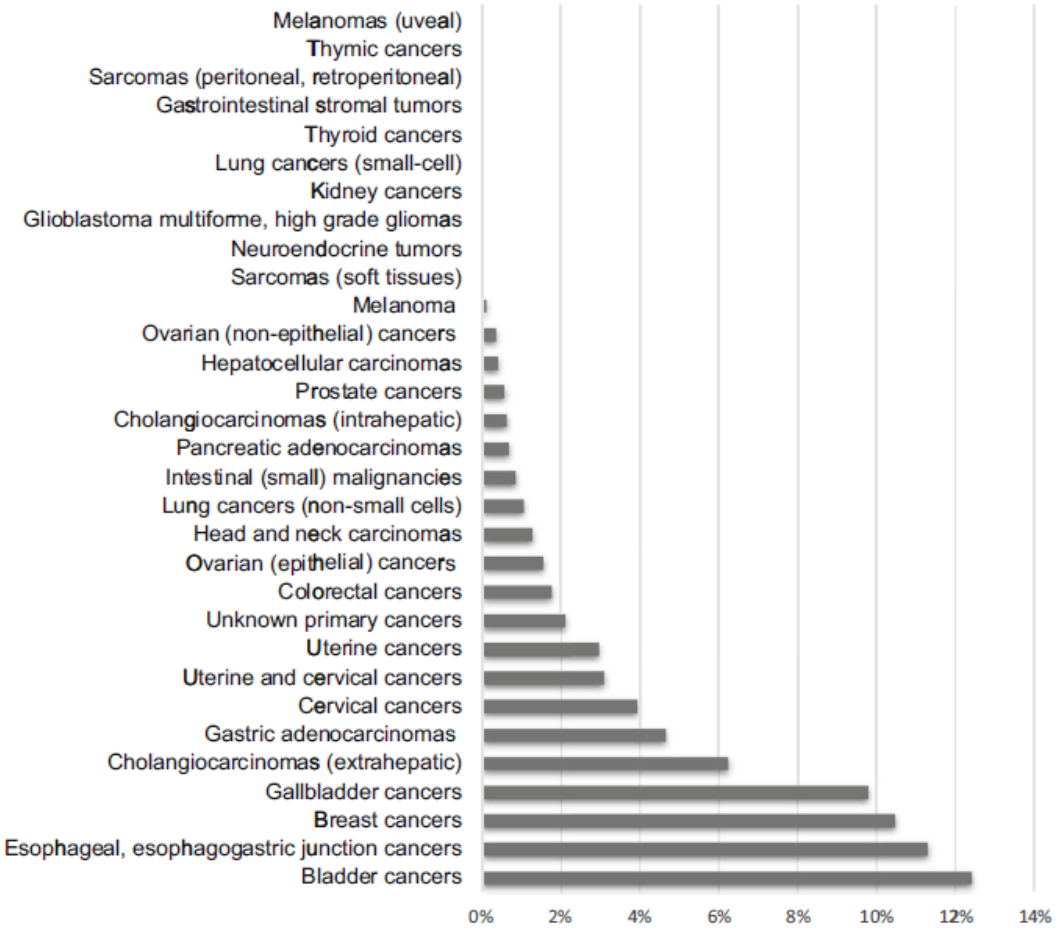
Heterogeneity

He Zhang et al., Pathology - Research and Practice (2020)

EUN YOON CHO et al., Pathology 2012

HER2 expression status in diverse cancers

Fig. 1 HER2 positivity across cancers: analysis of 37,992 samples by IHC. IHC 3+ was considered HER2 IHC positive



93 % were also positive by ISH

ISH Ratio >2: amplification

Min Yan et al., Cancer Metastasis Rev, 2015

In situ hybridization and its applications

Dr. S. Dedeurwaerdere
Department of pathology, AZ Delta, Roeselare

S. De Clercq, PhD
Department of pathology, Hôpital Erasme-ULB

In situ hybridization (ISH): part of the diagnostic process or as a predictive/prognostic marker

- Clinical data.
- HE and histochemistry.
- Immunohistochemistry.
- **ISH: chromogenic (CISH), silver (SISH), fluorescence (FISH).**
- Mutation analysis (NGS or other technique).
- Other: clonality analysis, hypermethylation assay,…

ISH data has to be correlated with other aspects of the diagnostic process!

You can use ISH in the setting of…

- **Diagnosis**: soft tissue tumors, renal tumors (e.g. FISH for TFE3 and TFEB), hematopathology (e.g. CISH EBV),…
- **Predictive testing**: HER2 SISH or FISH in breast cancer, ALK and ROS1 FISH in lung adenocarcinoma,…
- **Prognostic testing**: e.g. FISH for CMYC and BCL2/BCL6 translocation in “double hit” high grade B cell lymphoma.
- **Combinations of the above:** e.g. HPV DNA/RNA CISH in head & neck: predictive and prognostic (and diagnostic).

ISH is used for detection of….

- **Viral RNA or DNA**, e.g. EBV in hematopathology and HPV in head & neck and cervixpathology.
- **Numerical changes:**
  - **Gene region amplification**, e.g. MDM2 in liposarcoma, HER2 in breast.
  - **Chromosome polysomy**, e. g. trisomy 12 in CLL
  - **Loss of a gene/chromosome region (deletion),** e.g. Rb loss in spindle cell lipoma
- **Gene translocations** (break-apart FISH), e.g. translocation of EWSR1 in Ewing sarcoma.
- **Gene fusion**, e.g. EWSR1-FLI1 fusion in Ewing sarcoma

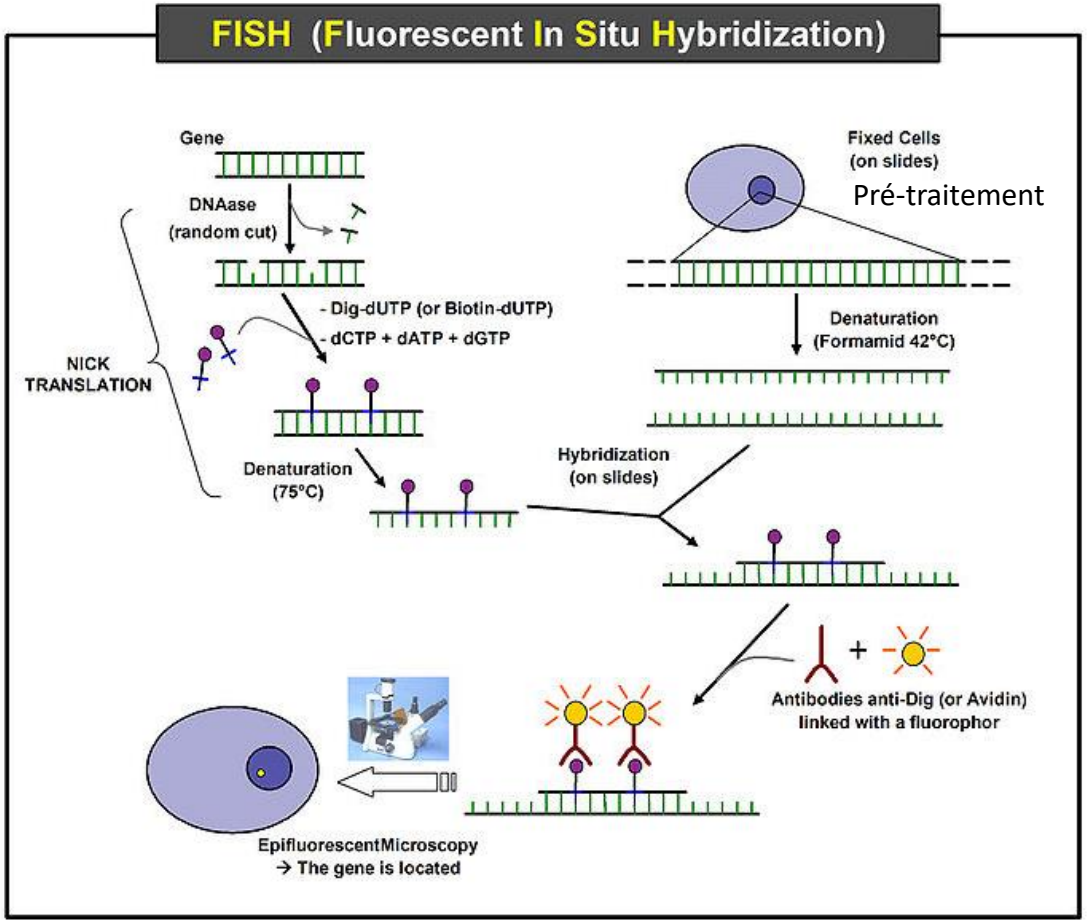
Hybridation *in situ*

L'Hybridation *in situ* (ISH) est une technique de biologie moléculaire qui permet l'étude des altérations de l'ADN dans des cellules tumorales par hybridation de sondes spécifiques sur coupes de tissus ou cellules fixées au formol et incluses en paraffine. Cette technique permet également l'identification de cellules exprimant de l'ARN de virus, tel que celui du virus d'Epstein-Barr.

- Amplification de gènes
- Délétion de gènes
- Réarrangements de gènes
- Détection d'ARN viraux

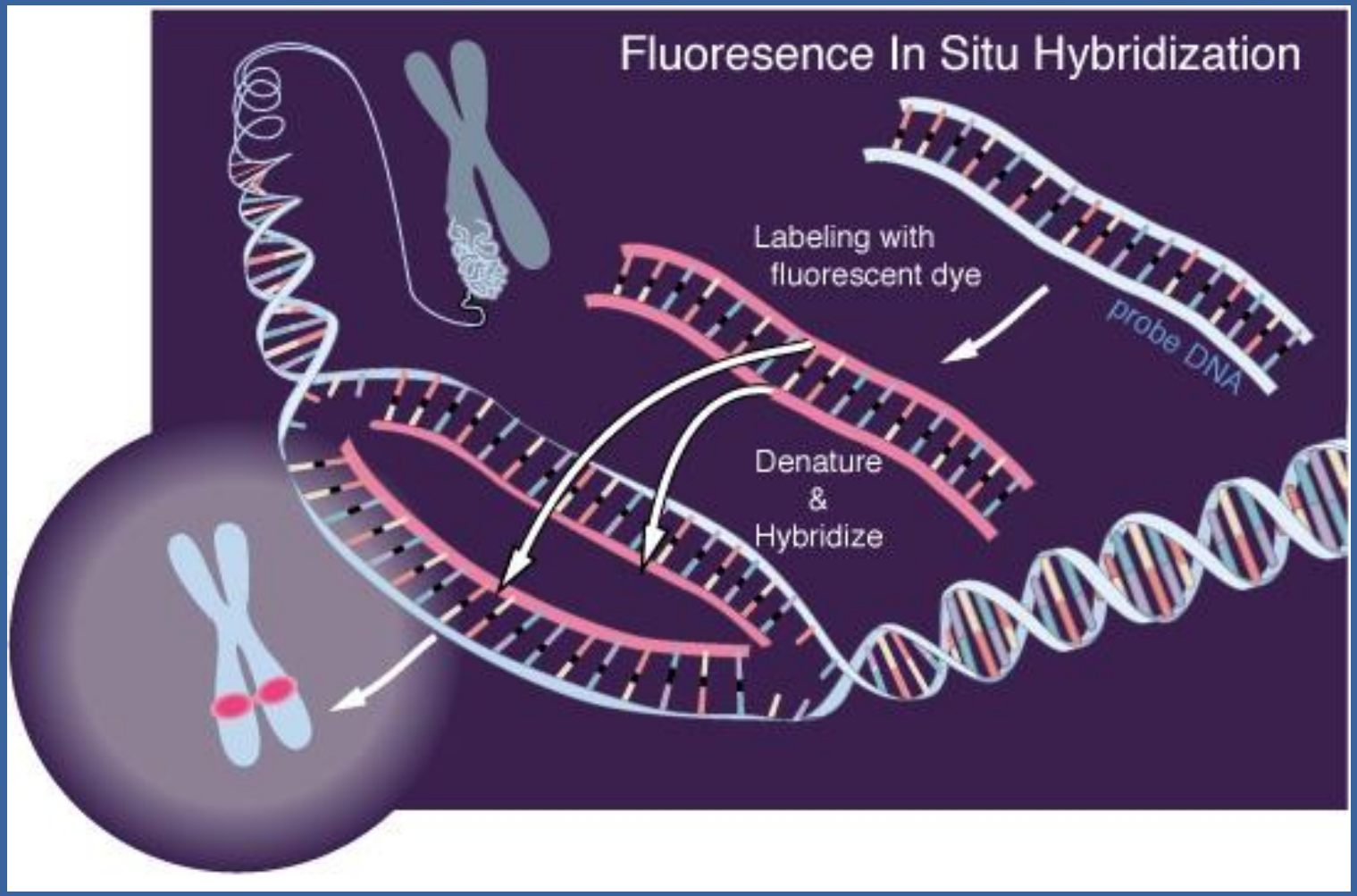
FISH: Fluorescent In Situ Hybridization

CISH: Chromogenic In Situ Hybridization



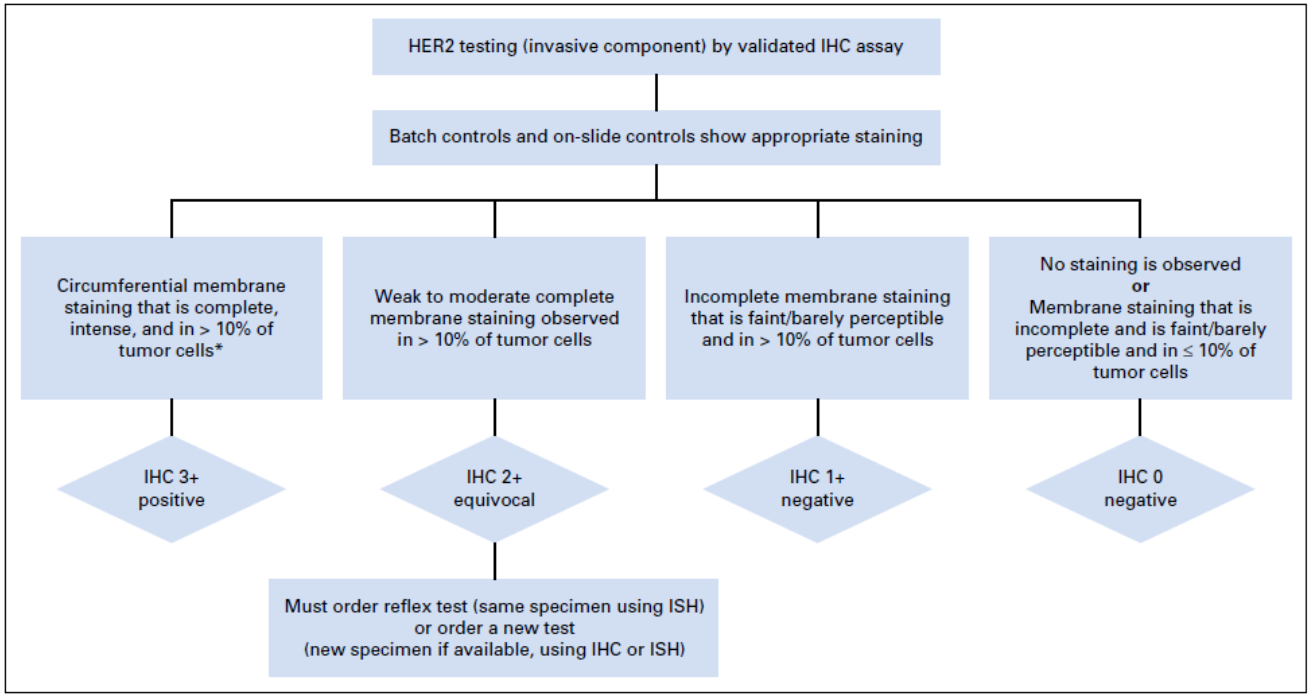
Basic principles of the ISH technique

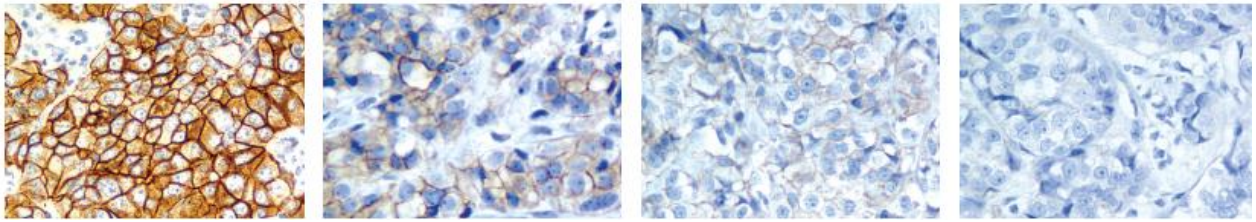
- Tissue sectioning.
- Heating/denaturation.
- Probe links with DNA/RNA target sequence of interest.
- Probe is (usually directly) visualised via fluorophore in FISH and (usually indirectly, using antibodies) via chromogens in CISH and SISH.
- Counterstain (blue), of only nuclei in FISH and of overall tissue in CISH/SISH.


Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize

IHC

Wolff et al., J Clin Oncol 2018: 36; 2105-2122.

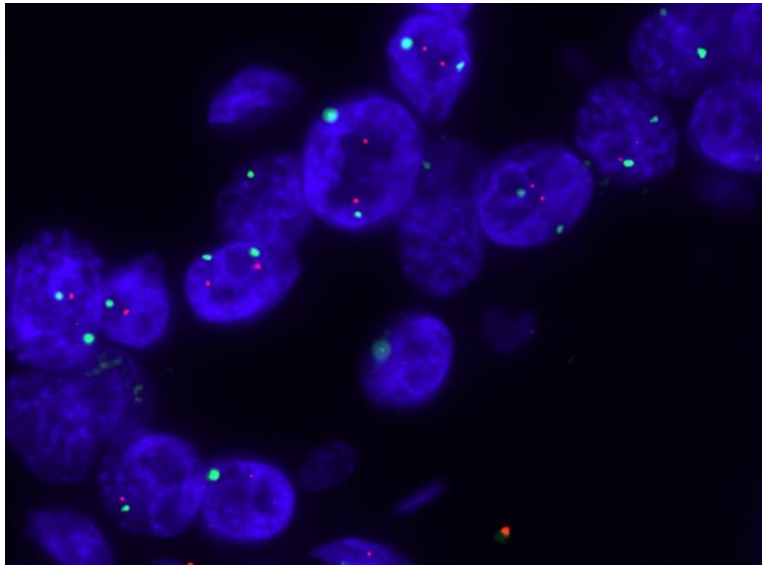




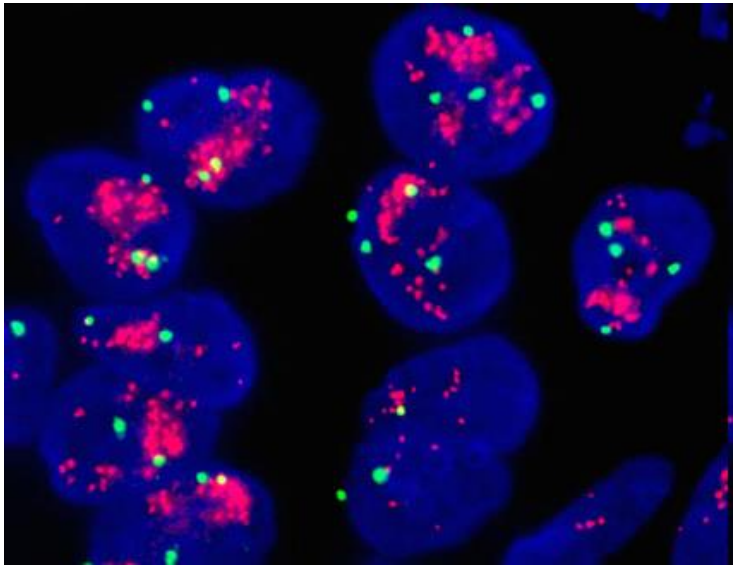
FISH

FISH HER2

Nombre de noyaux analysés : 20
Nombre moyen de copies de centromère 17 par cellule.
Nombre moyen de copies de HER2 par cellule.
Rapport HER2 / centromère 17.



Absence d'amplification



Amplification Her2

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, Mitchell Dowsett

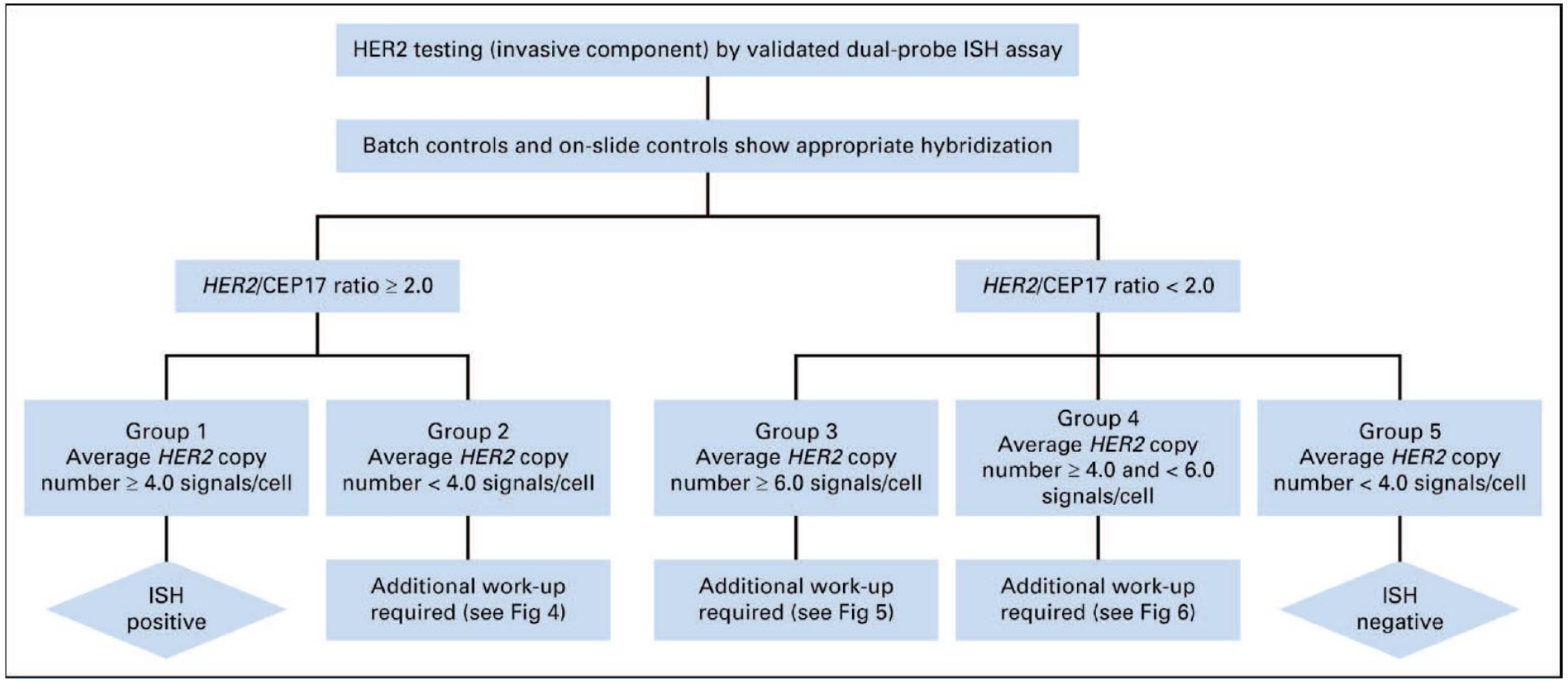


Figure 4

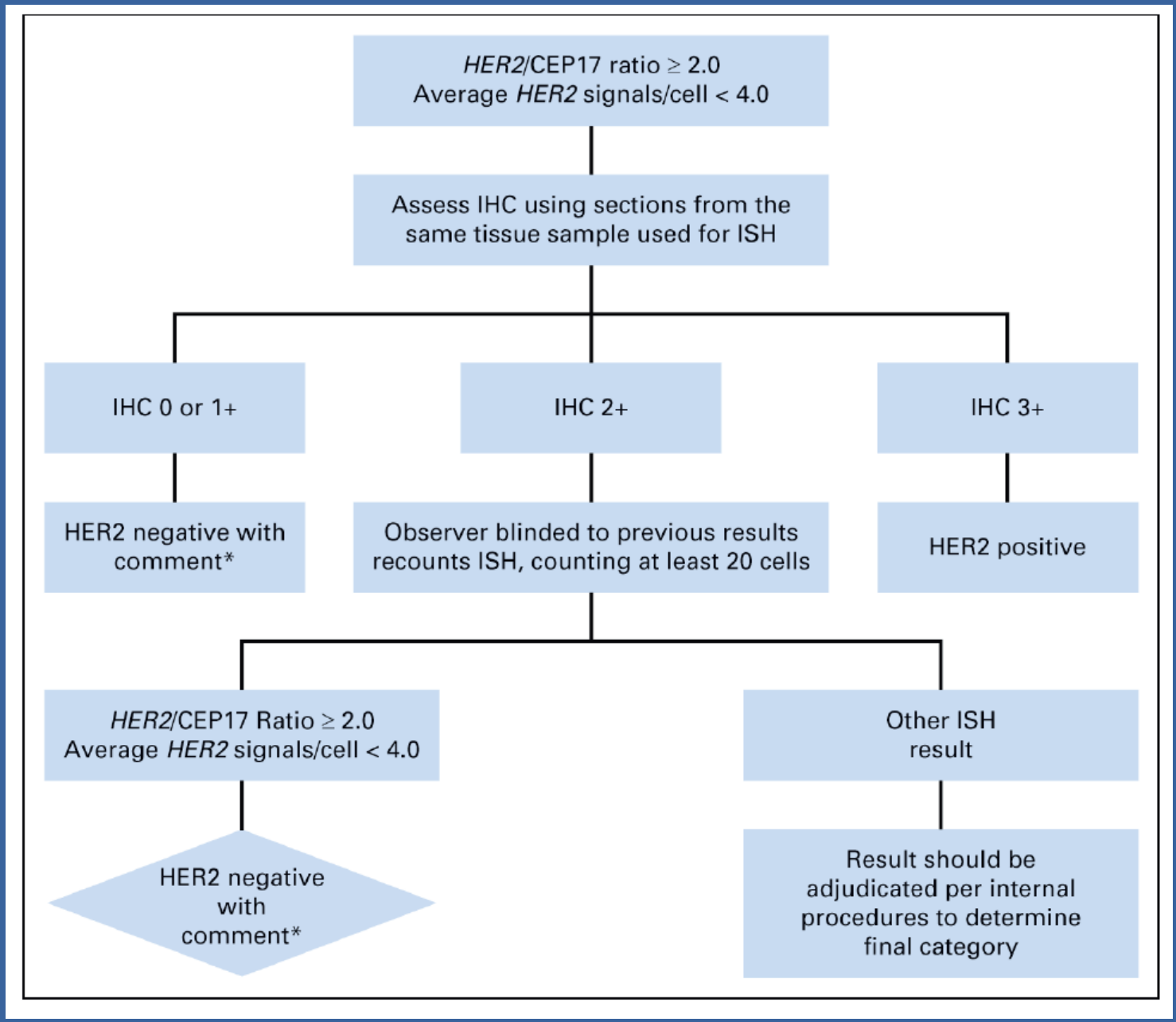

HER2/CEP17 ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 Ratio ≥ 2.0
Average *HER2* signals/cell < 4.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

Figure 5

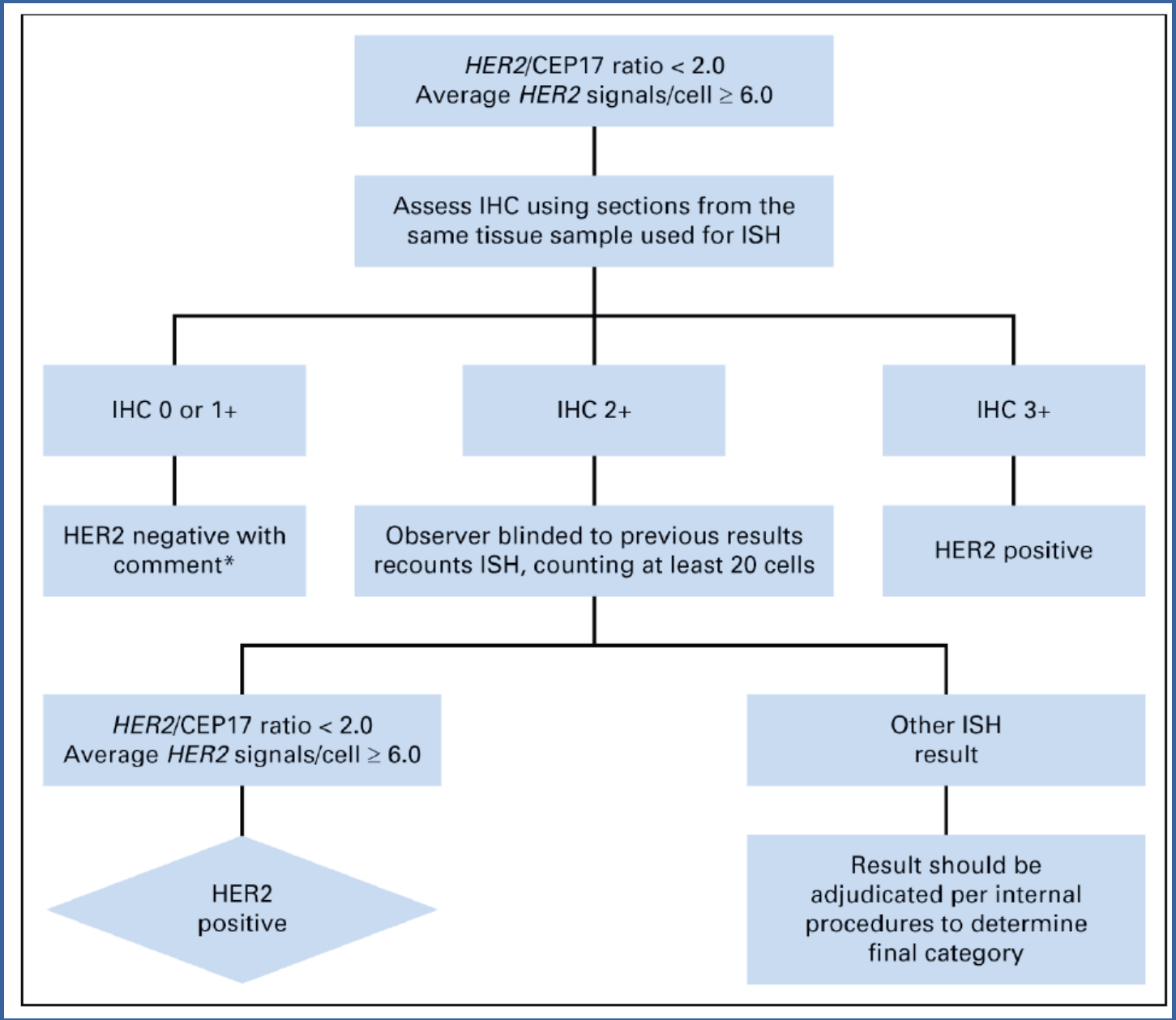
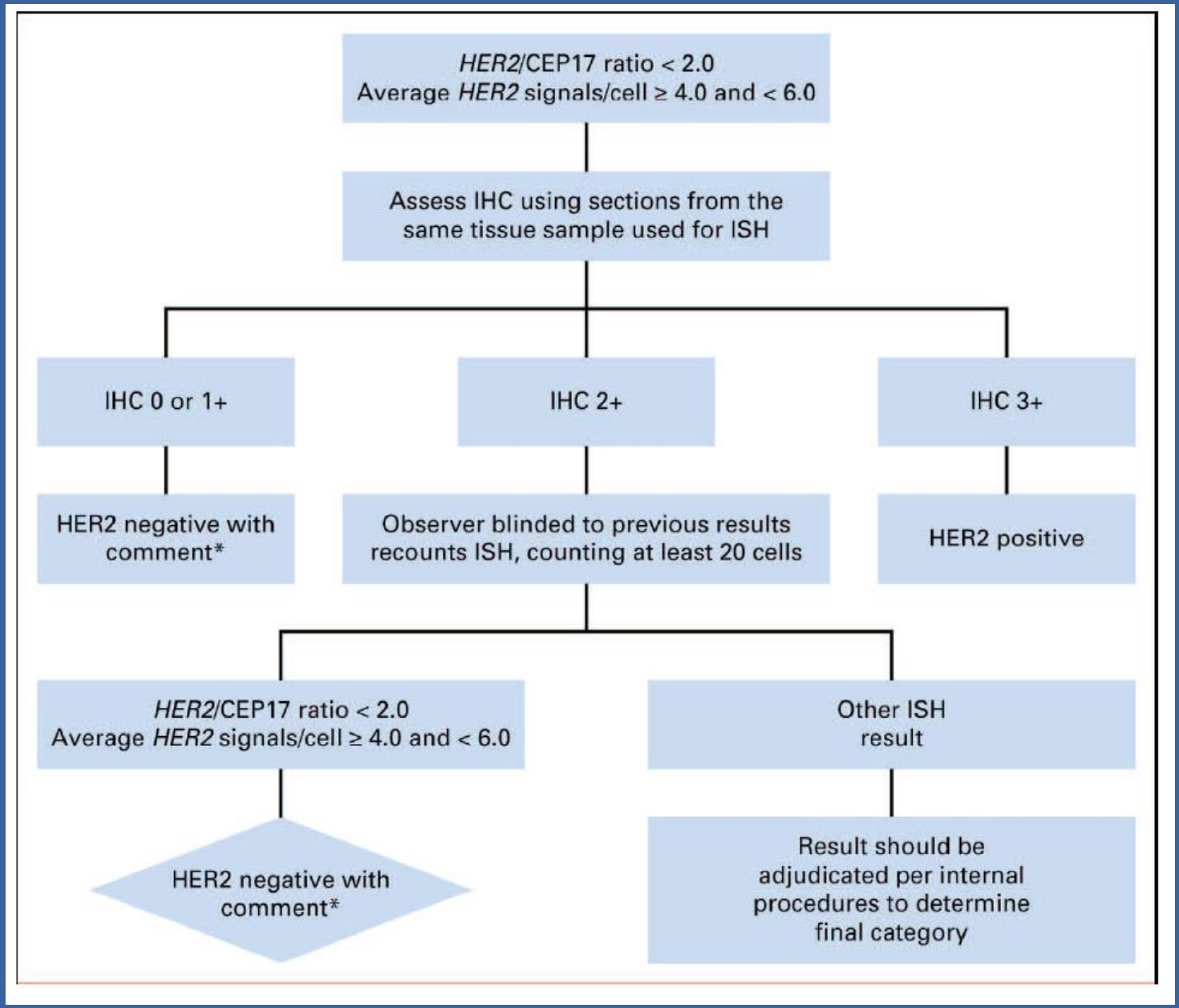

HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 6.0
Other ISH result
HER2 positive
Result should be adjudicated per internal procedures to determine final category

Figure 6


HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 4.0 and < 6.0
Assess IHC using sections from the same tissue sample used for ISH
IHC 0 or 1+
IHC 2+
IHC 3+
HER2 negative with comment*
Observer blinded to previous results recounts ISH, counting at least 20 cells
HER2 positive
HER2/CEP17 ratio < 2.0
Average *HER2* signals/cell ≥ 4.0 and < 6.0
Other ISH result
HER2 negative with comment*
Result should be adjudicated per internal procedures to determine final category

FISH HER2 RÉSUMÉ

Ratio	HER2/CEN17 ratio ≥ 2		HER2/CEN17 ratio < 2		
Nombre moyen de signaux HER2 par cellule	≥ 4	< 4	≥ 6	≥ 4 et < 6	< 4
Résultat	**Amplification**	dépendant du score IHC HER2			**Absence d'amplification**

Résultat si IHC 2+	**Absence d'amplification*[1]**	**Amplification**	**Absence d'amplification*[2]**
Résultat si IHC 3+	**Amplification*[3]**	**Amplification**	**Amplification*[4]**

Wolff et al., J Clin Oncol 2018: 36; 2105-2122.

- ***[1] Commentaire :** L'efficacité des traitements ciblant HER2 dans les rares cas présentant un ratio HER2/CEN-17 ≥ 2 et ayant un nombre moyen de signaux HER2 par cellule inférieur à 4 n'a pas encore été démontrée. En l'absence d'un score IHC 3+ et vu le faible nombre de copies du gène HER2, il est recommandé de considérer ces cas comme négatifs. *Wolff et al., J Clin Oncol 2018: 36; 2105-2122.*
- ***[2] Commentaire :** Il n'est pas encore certain que les cas ayant un nombre moyen de signaux HER2 par cellule compris entre ≥ 4 et < 6 et un ratio < 2 répondent aux traitements ciblant HER2 en l'absence d'un score IHC 3+. Il est donc recommandé de considérer ces cas comme négatifs. *Wolff et al., J Clin Oncol 2018: 36; 2105-2122.*
- ***[3] Commentaire :** L'efficacité des traitements ciblant HER2 dans les rares cas présentant un ratio HER2/CEN-17 ≥ 2 et ayant un nombre moyen de signaux HER2 par cellule inférieur à 4 n'a pas encore été démontrée. Cependant, étant donné le score IHC 3+, les guidelines recommandent de considérer ces cas comme amplifiés. *Wolff et al., J Clin Oncol 2018: 36; 2105-2122.*
- ***[4] Commentaire :** Il n'est pas encore certain que les cas ayant un nombre moyen de signaux HER2 par cellule compris entre ≥ 4 et < 6 et un ratio < 2 répondent aux traitements ciblant HER2 en l'absence d'un score IHC 3+. En présence d'un score IHC 3+, les guidelines recommandent de considérer ces cas comme amplifiés. *Wolff et al., J Clin Oncol 2018: 36; 2105-2122.*

Belgian guidelines for HER2 testing in gastric cancer

A.Jouret-Mourin, A. Hoorens M. Kockx, P. Demetter, E. Van Cutsem

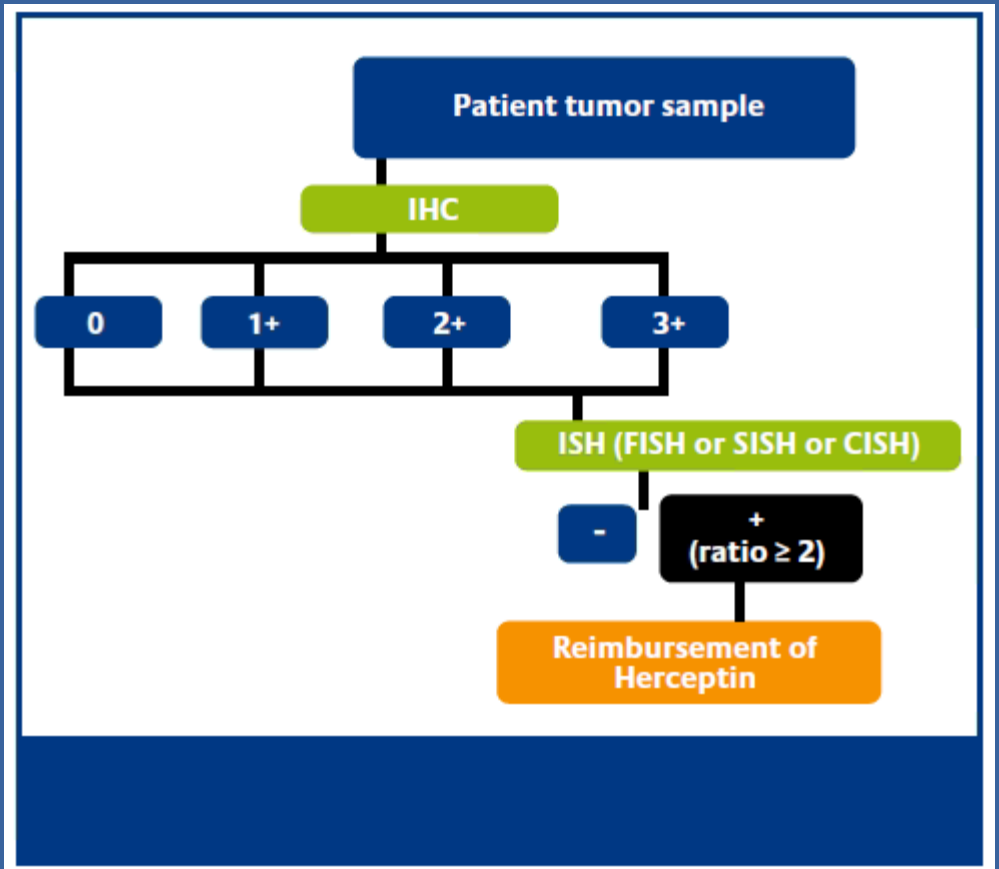
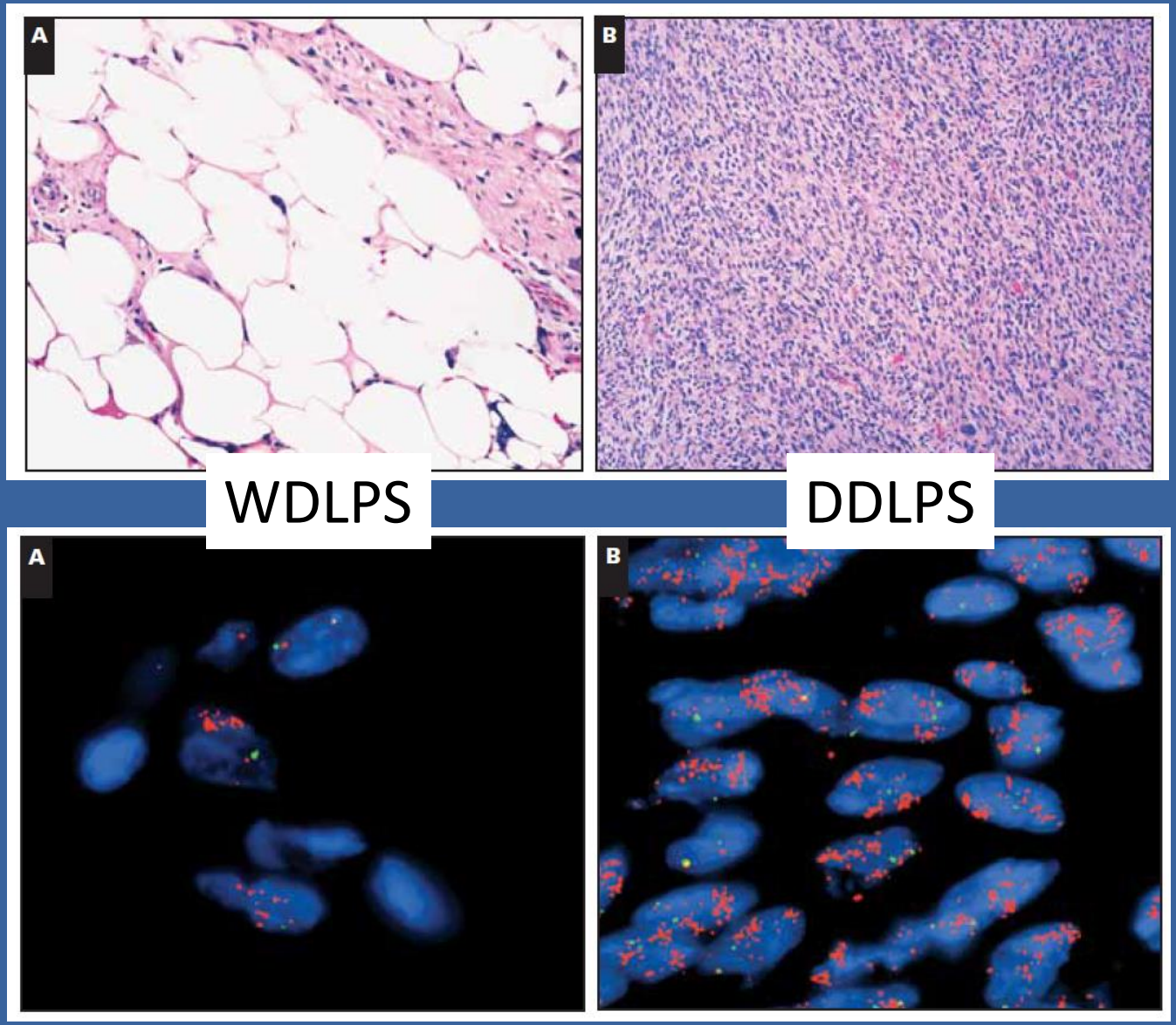


Figure 4. Belgian algorithm for HER2 testing in gastric cancer.

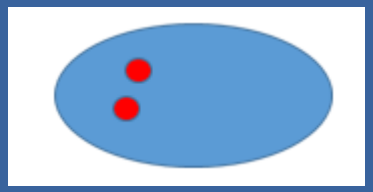
MDM2 is amplified in liposarcoma and copy number increases from WDLPS to DDLPS

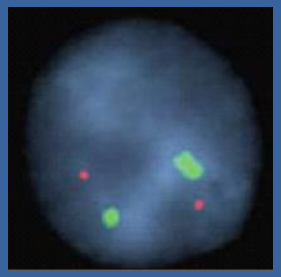


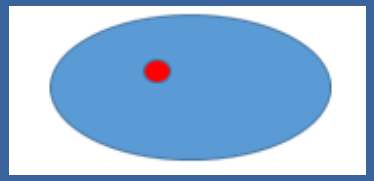
Ware et al. AJCP 141:334

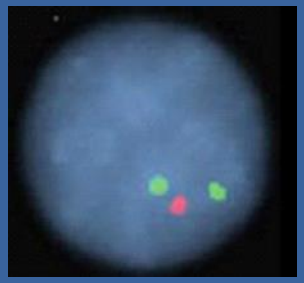
Numerical changes: polysomy/deletion

Deletion, e.g.TP53 in CLL

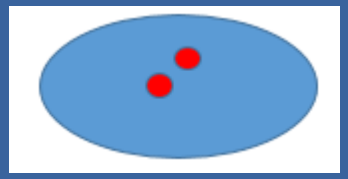


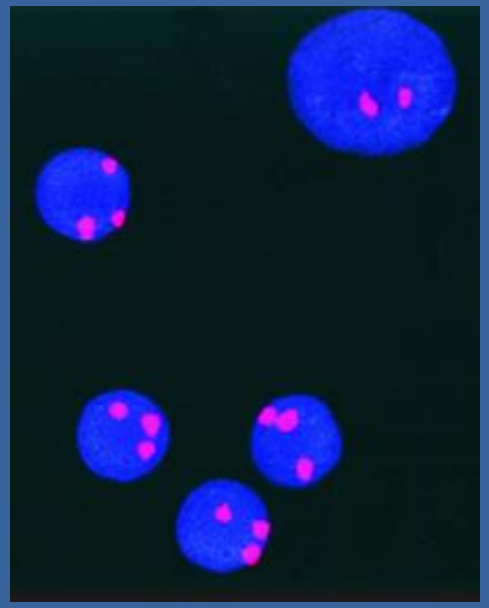


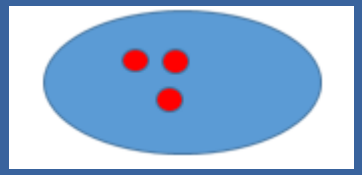




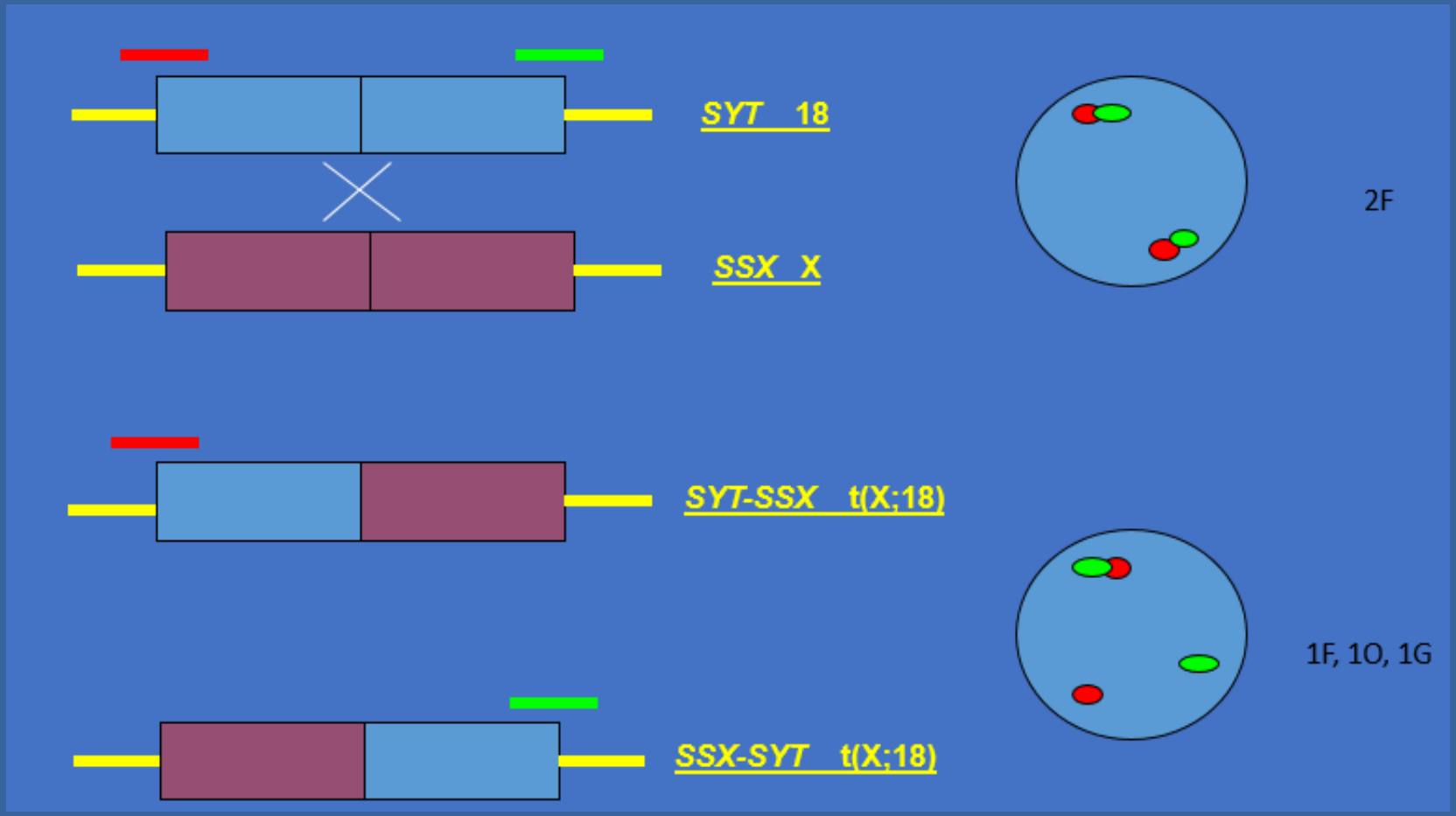
Trisomy, e.g.trisomy 12 in CLL





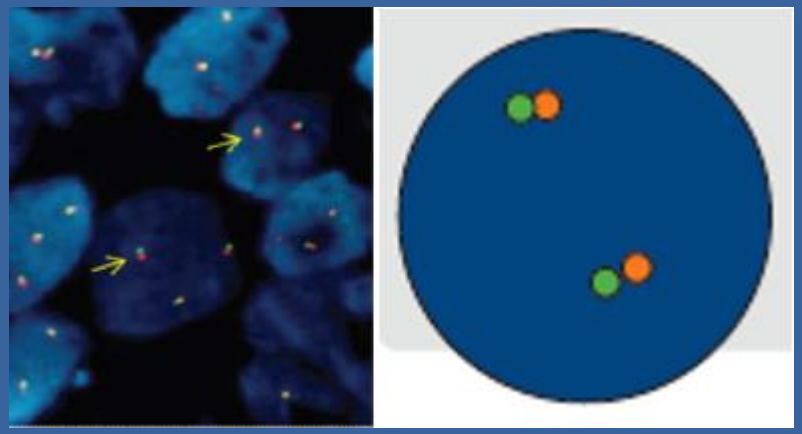


Detection of gene translocations using break apart probes



Lung adenocarcinoma: ALK and ROS1

Normal pattern

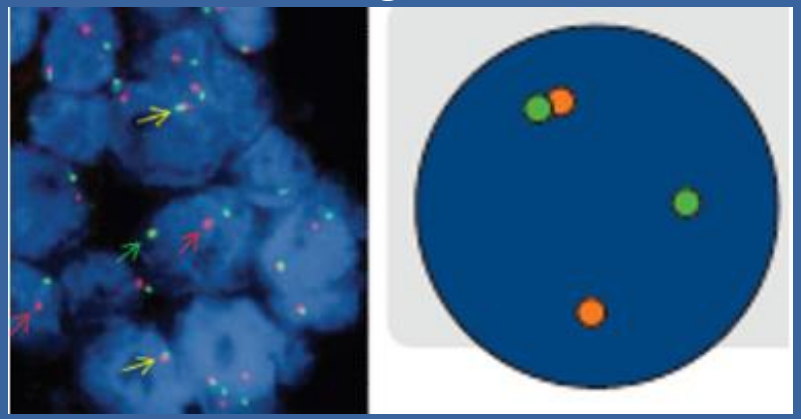


2 fused signals

ALK

ROS1

ALK/ROS1 rearrangements



1 fused signal and 1 split signal

Isolated 3’ signal pattern and 1 fused signal

Lung adenocarcinoma: ALK and ROS1

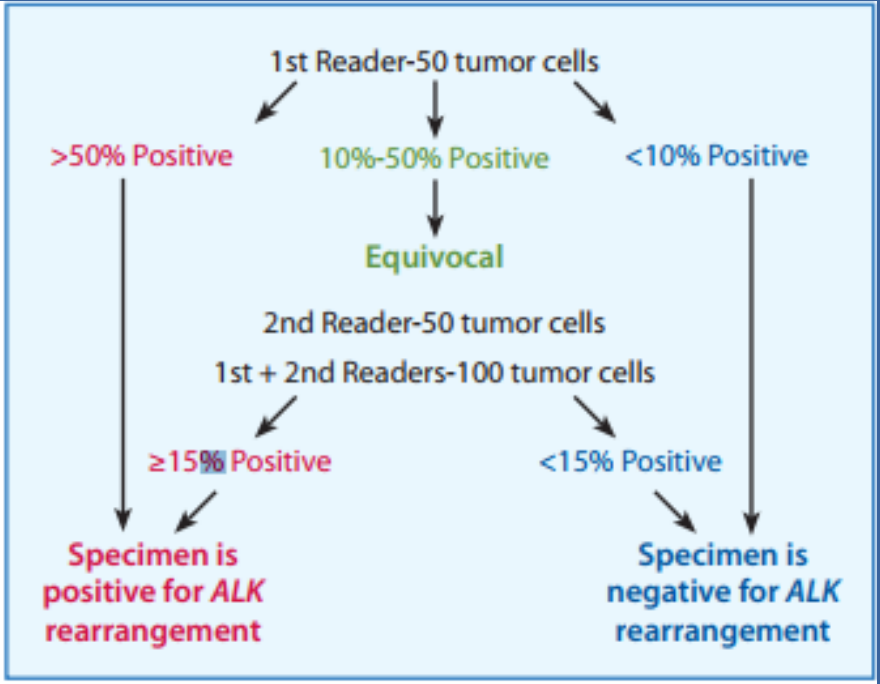


Figure 11. Recommended scoring algorithm for ALK FISH.

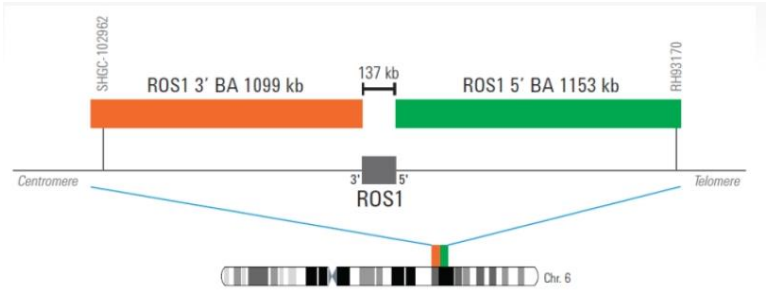
Pathological diagnosis and molecular testing in non-small cell lung cancer: Belgian guidelines

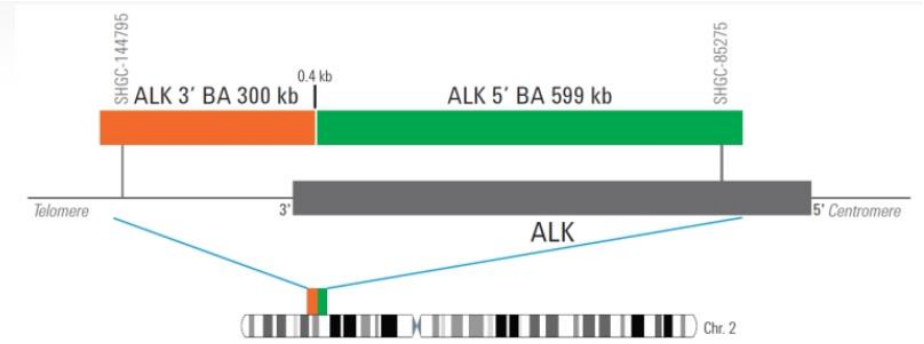
P. Pauwels, MD, PhD[1], M. Remmelink, MD, PhD[2], D. Hoton, MD[3], J. Van Dorpe, MD, PhD[4], K. Dhaene, MD, PhD[5], F. Dome, MD[6], A. Jouret-Mourin, MD, PhD[3], B. Weynand, MD, PhD[7], N. D'Haene, MD, PhD[2]

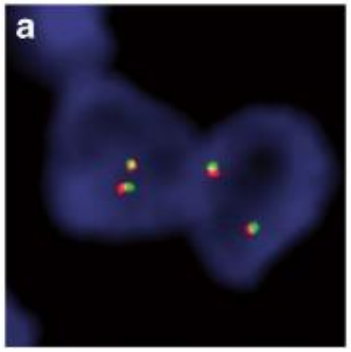
On behalf of the Working Group of Molecular Pathology and the Belgian Society of Pathology

ALK ET ROS1 FUSION

Break apart probes, designed to be on opposite sides of the translocation break point for a given gene, each labeled in a different color.

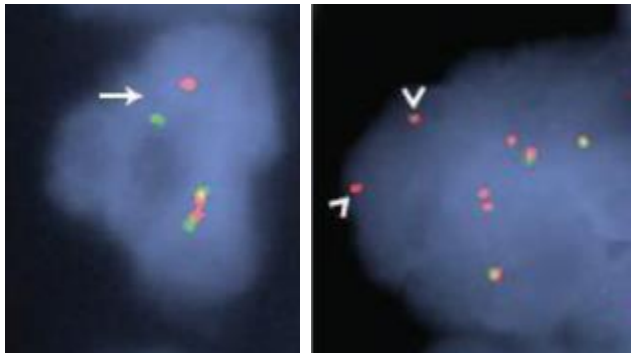






Normal pattern

2 fused signals



ALK/ROS1 rearrangements

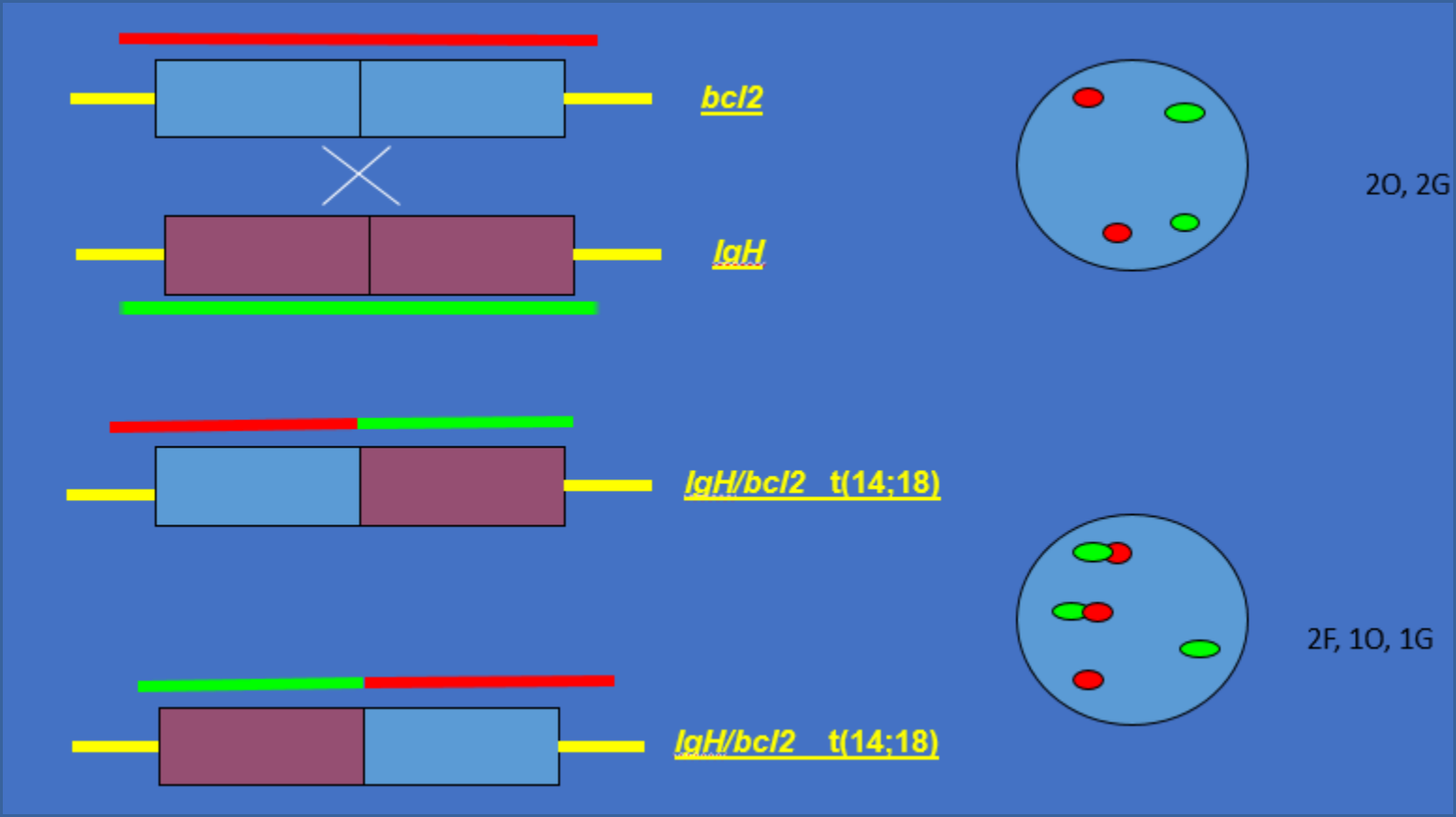
-1 fused signal and 1 split signal

- Isolated 3’ signal pattern and 1 fused signal

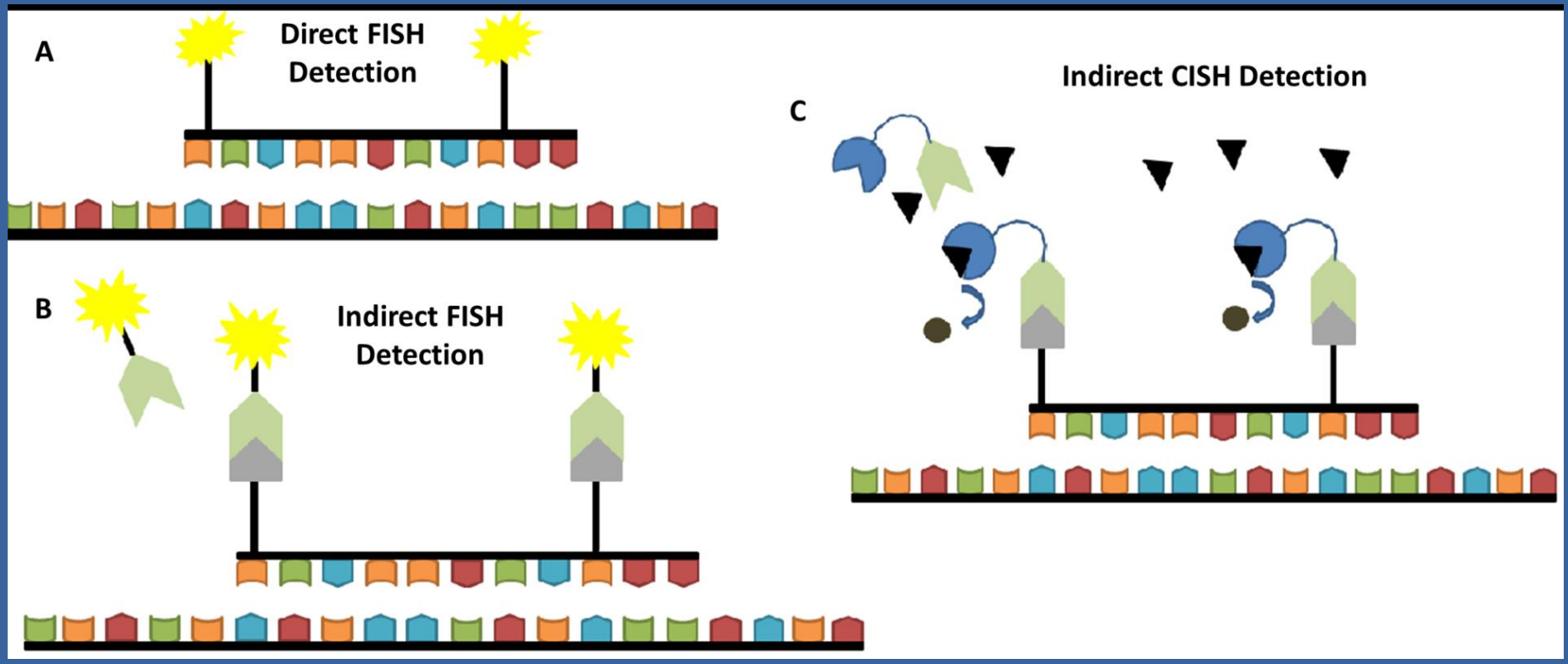
	Positivity criteria	
Number of cells counted	At least 50 tumour cells (first step); 100 cells (second step)	
Patterns for positivity	Typical pattern: two separated 3′ and 5′ plus one fusion signal; Atypical pattern: isolated 3′ signal plus one fusion signal	
	First step	
Score of positivity	25 positive cells out of 50 tumour cells	>50%
Negativity	Less than 5 positive tumour cells	<10%
Equivocal	5–25 positive cells (need second observer for an additional cell count reading)	
	Second step (for an equivocal result)	
Positivity threshold (additional cell count reading)	≥15 % positive cells out of 100 tumour cells	

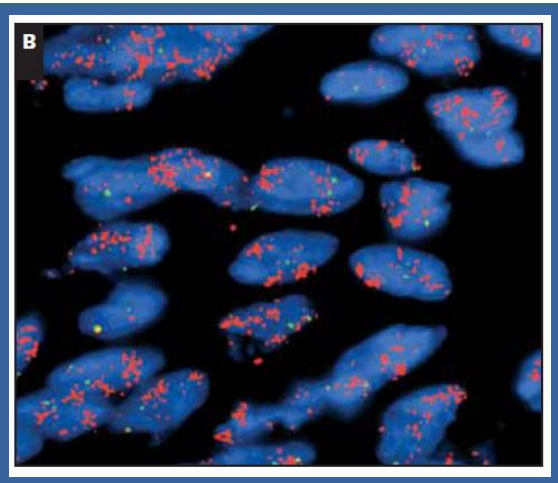
Peters et al., Lung Cancer, 2013.
von Laffert et al., Lung Cancer, 2015.
Bubendorf et al., Virchows Arch., 2016.

Detection of gene fusions using fusion probes

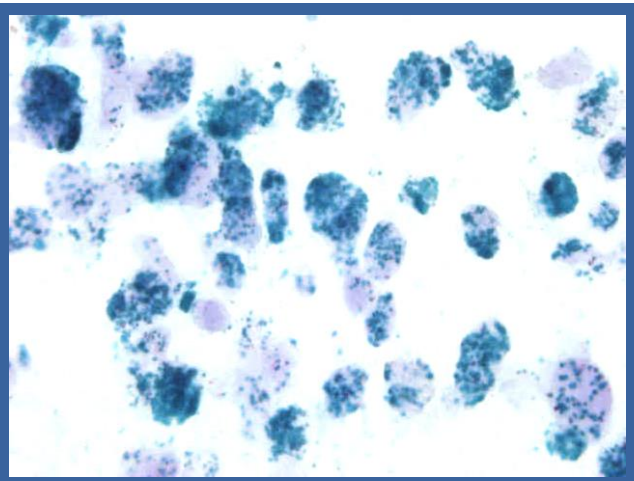


FISH versus CISH

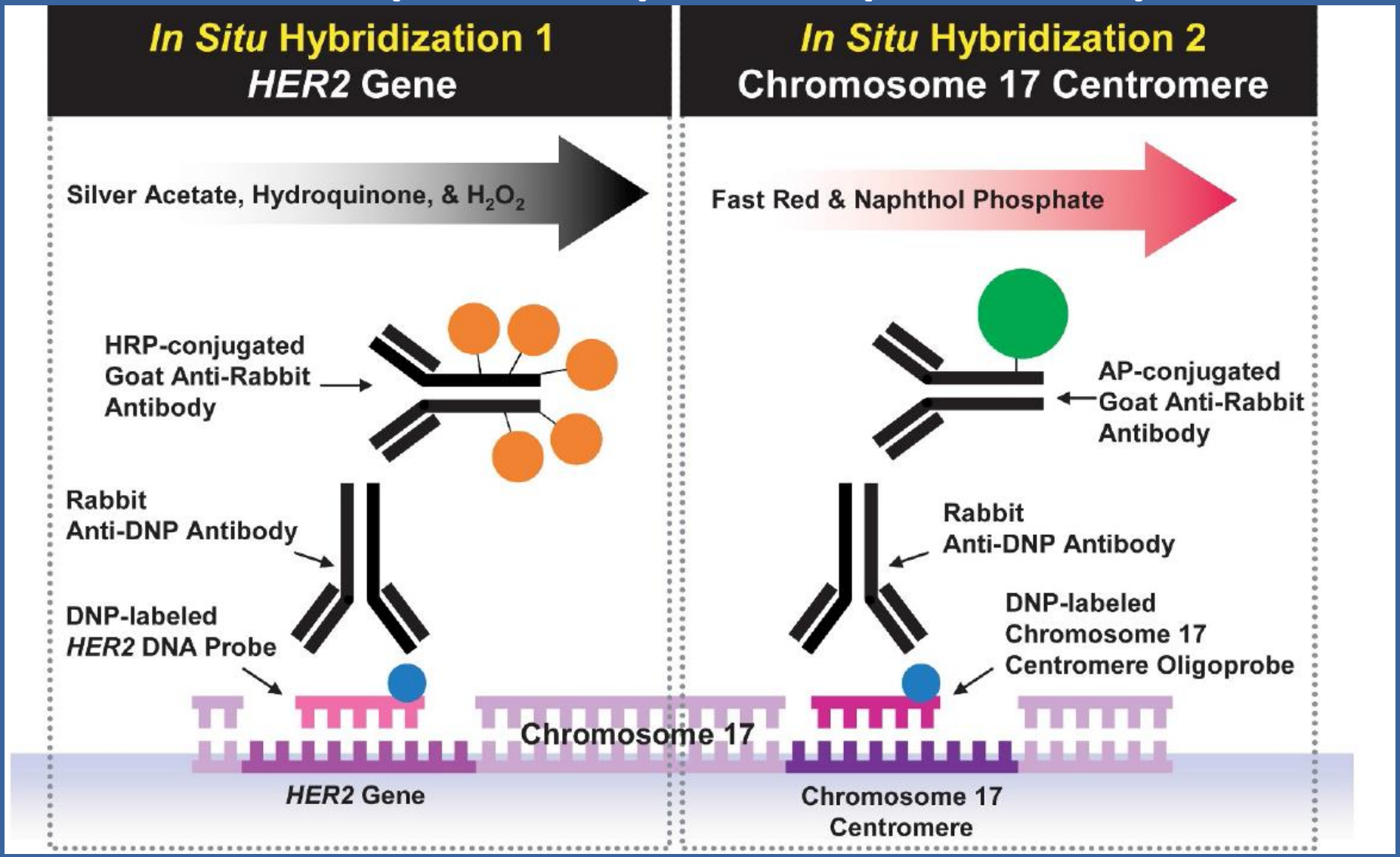




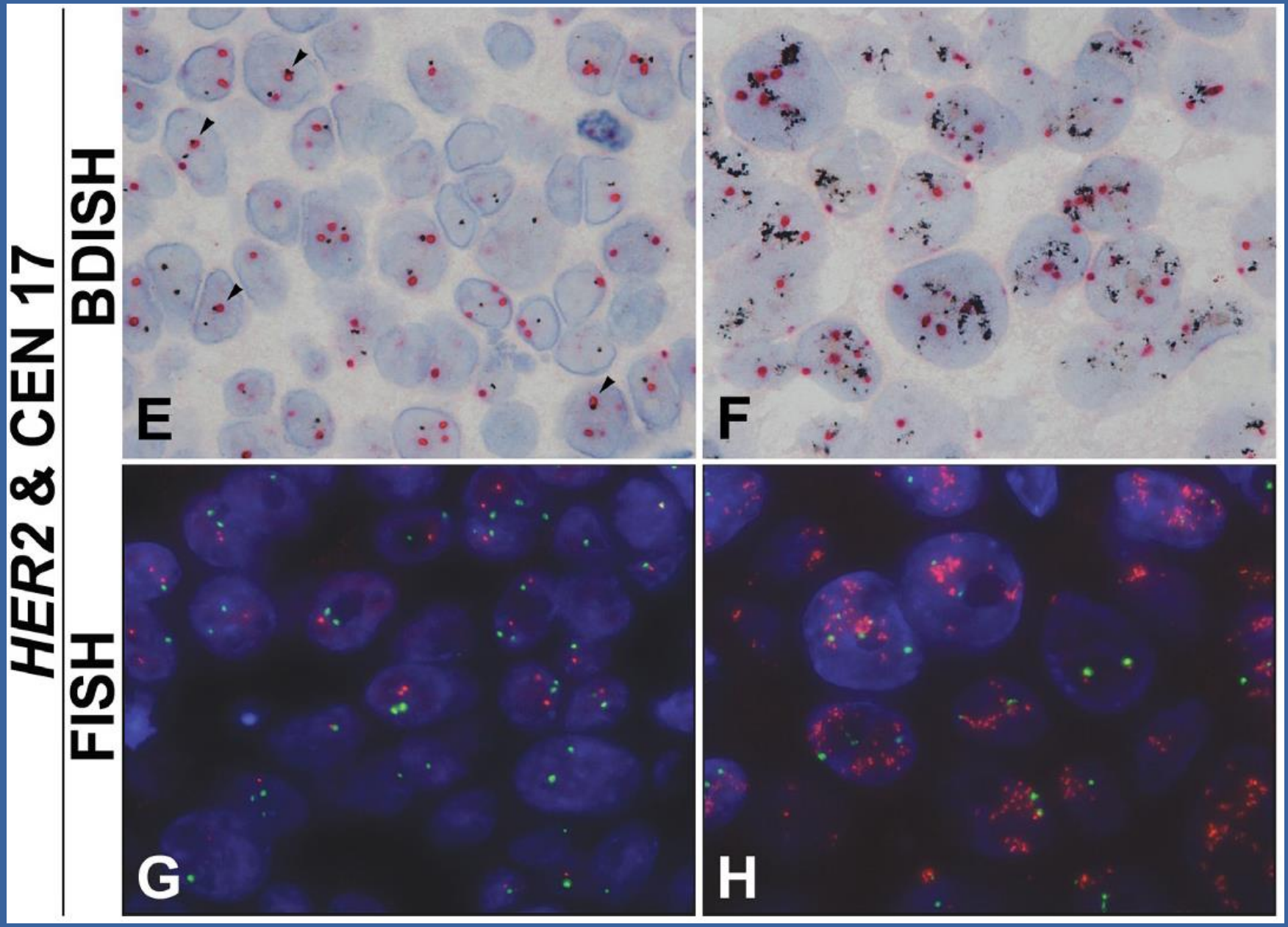
Mdm2
amplification



Bright-field double in situ hybridization (BDISH): SISH (for HER2) + CISH (for CEP17)



Nitta et al. Diagn Pathol 3:41



Nitta et al. Diagn Pathol 3:41

Advantages of FISH over CISH and SISH

- Higher resolution (more « pixels » per surface area).
- Therefore, translocations and fusions of genes can only be evaluated using FISH.
- Test failure is rarer in FISH compared to SISH.

Advantages of SISH/CISH over FISH

- Signals of S/CISH do not fade; slides can always be reevaluated, while FISH fades after a few months.

- S/CISH is more practical: normal microscope can be used, not needed to work in a dark room.

- In S/CISH you see the background cellular and architectural structure: you see where you are. In FISH, only nuclei are seen.

Specific topics regarding FISH in soft tissue tumors

- FISH is a very powerful tool to solve a DD and to obtain unequivocal confirmation of a suspected diagnosis.

- There is no agreement regarding cut-offs, e.g. some consider 10% of tumoral cells with EWSR1 translocation as positive, other require up to 30%.

- A lot of genes are “promiscuous”: they can have different fusion partners.

- Sometimes, more than 1 FISH is needed and combination of break-apart and fusion FISHes can be necessary: time-consuming and cumbersome.

- RT-PCR for mRNA fusion: rarely used, frozen tissue is needed. Does not solve the issue.

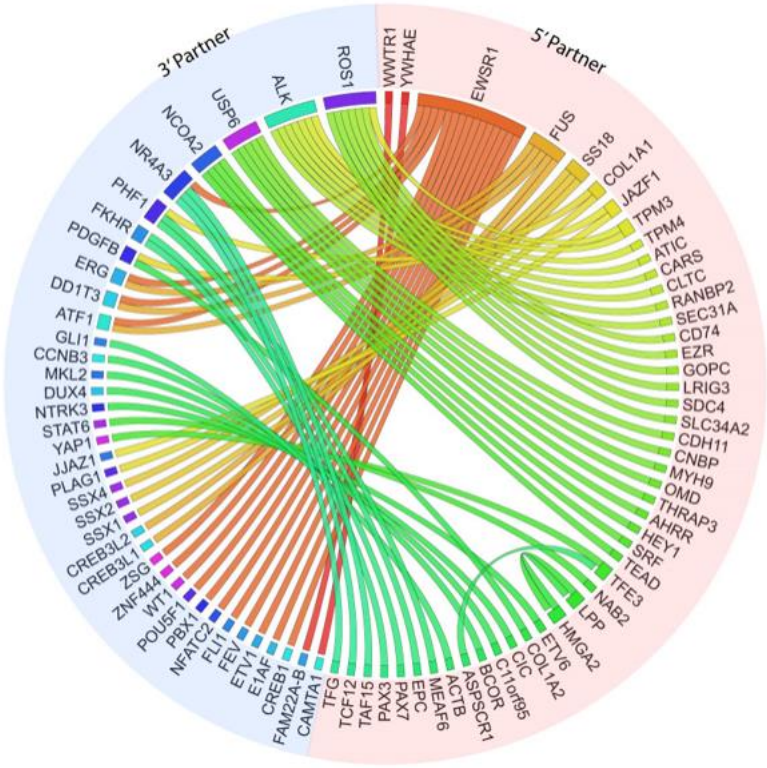
- Detection of a high number of fusion genes in 1 test on FFPE, as in NGS for mutations: fusion panels are the solution?

Archer® FusionPlex® Sarcoma Kit

Assay Targets

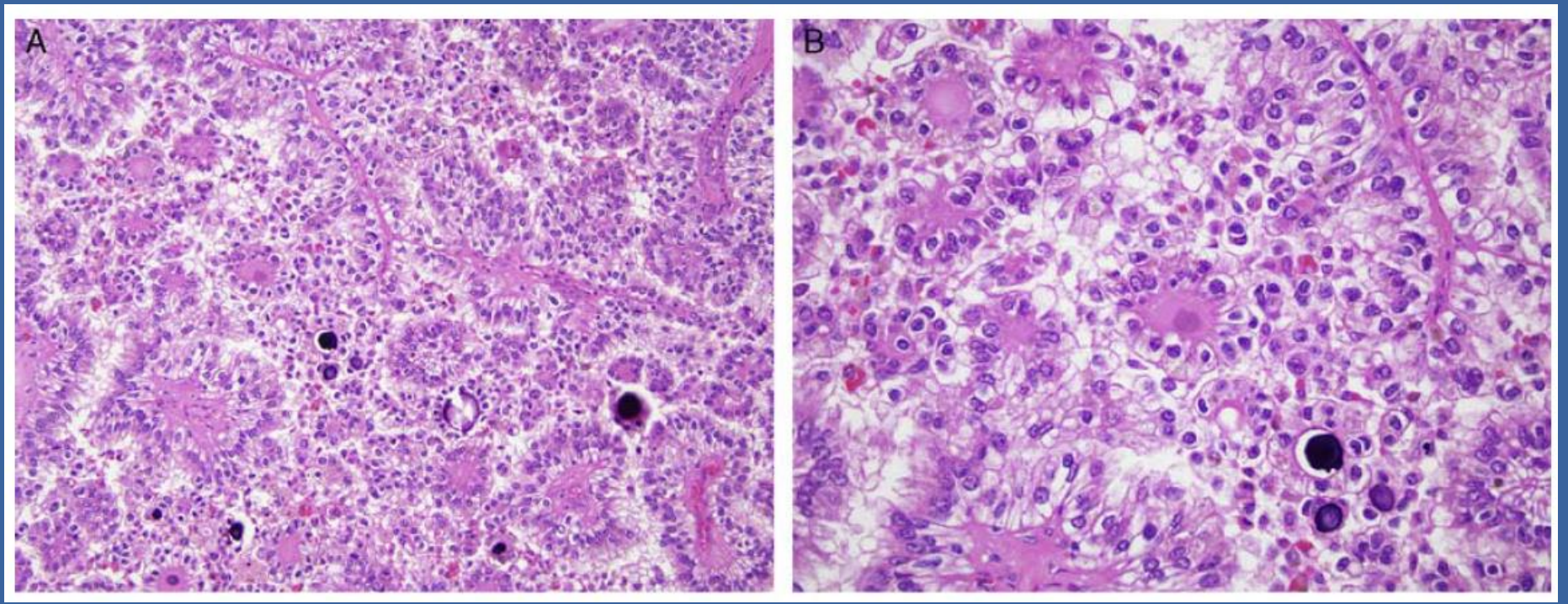
ALK	CAMTA1	CCNB3	CIC
EPC	EWSR1	FKHR	FUS
GLI1	HMGA2	JAZF1	MEAF6
MKL2	NCOA2	NTRK3	PDGFB
PLAG1	ROS1	SS18	STAT6
TAF15	TCF12	TFE3	TFG
USP6	YWHAE		

Sarcoma Gene Fusion Map

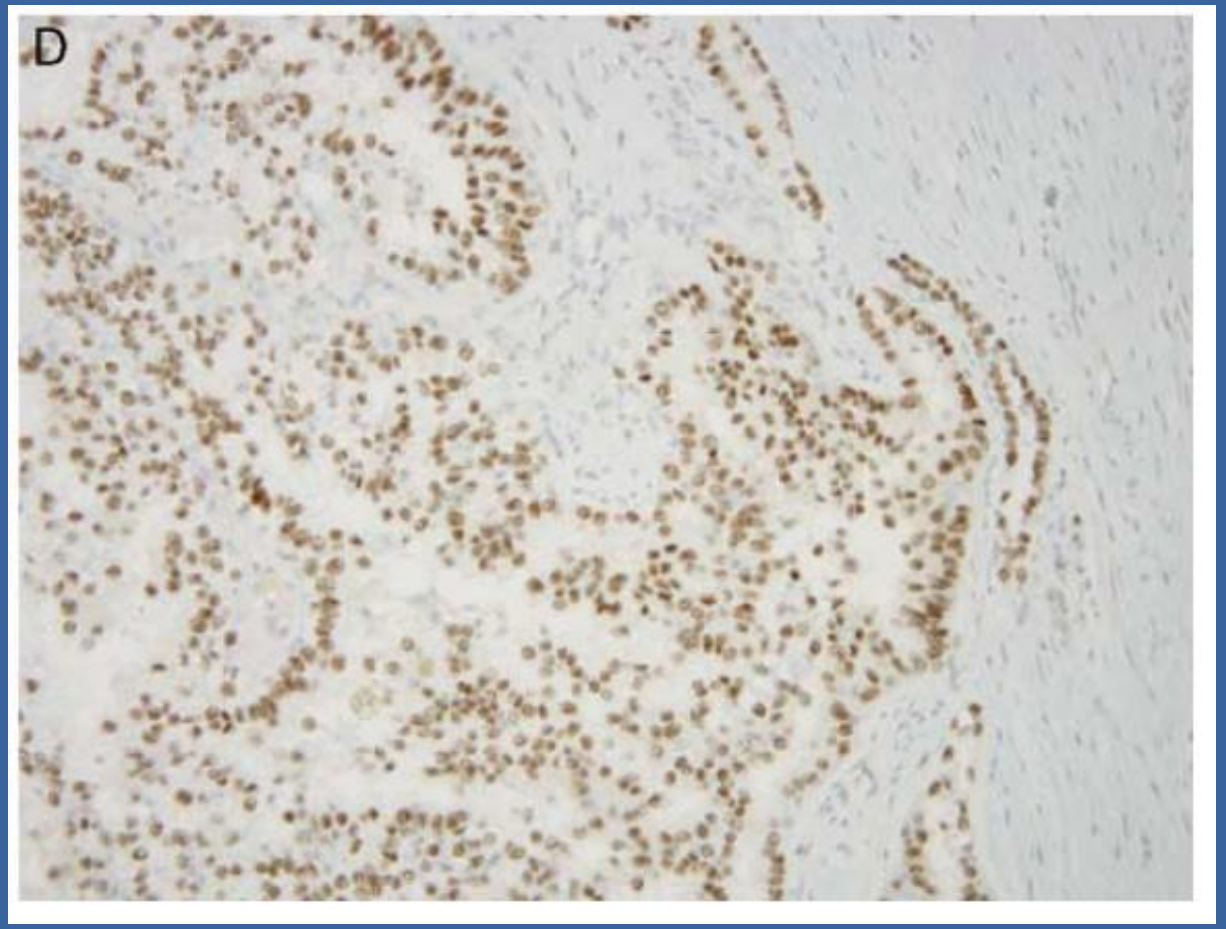


This schematic shows known soft tissue sarcoma gene fusions. Everything shown is detected by this assay.

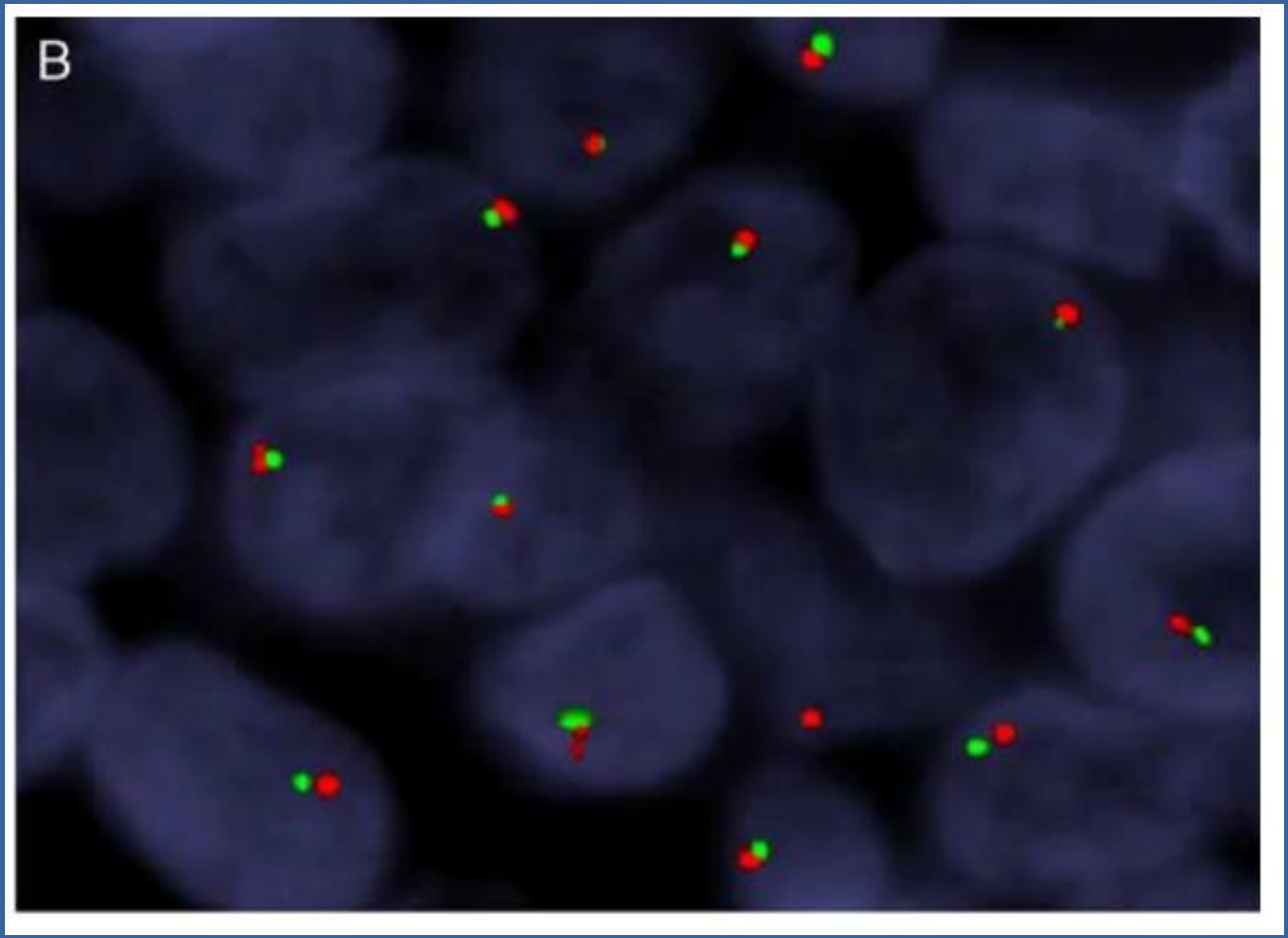
And now the final topic:



A renal cell carcinoma with papillary structures, clear cells and psammoma bodies:
HE strongly suggestive for
TFE3 translocation renal cell carcinoma



TFE3 IHC diffusely positive



TFE3 FISH:

red and green signal together, so TFE3 is not displaced

So TFE3 IHC is false positive and does not reflect a translocation of the gene?

So this is not a TFE3 translocation renal carcinoma?

RBM10-TFE3 Renal Cell Carcinoma

A Potential Diagnostic Pitfall Due to Cryptic Intrachromosomal Xp11.2 Inversion Resulting in False-negative TFE3 FISH

Pedram Argani, MD,† Lei Zhang, MD,‡ Victor E. Reuter, MD,‡ Satish K. Tickoo, MD,‡ and Cristina R. Antonescu, MD‡*

- In this tumor, the partner of TFE3 is RBM10.
- RBM10 is normally only 1.8 Mb away from TFE3.
- So, TFE3 “moves” only a very little bit, via inversion.
- On a normal break-apart FISH, this subtle move can not be seen; a specific RBM10-TFE3 FISH was positive.

AJSP 41:655

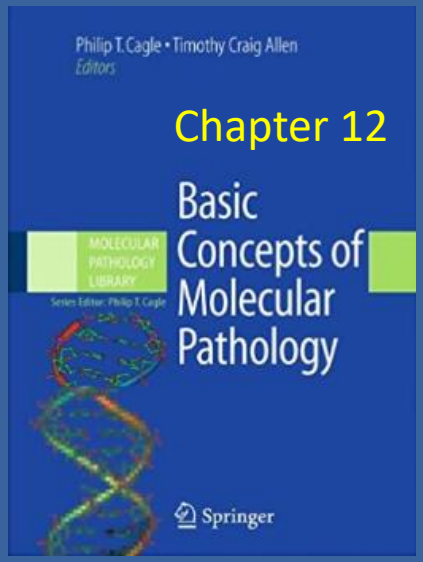
So, IHC TFE3 was not false positive

The TFE3 FISH was false negative

A gene rearrangement can be cryptic on FISH (for several reasons)

Always remain critical and open-minded (not only concerning FISH-issues)

Course material



Fluorescent In Situ Hybridization in Surgical Pathology Practice

Gupta, Ruta, FRCPA[*,†]; Cooper, Wendy, A., PhD, FRCPA[*,†,‡]; Selinger, Christina, PhD[*]; Mahar, Annabelle, FRCPA[*]; Anderson, Lyndal, FRCPA[*,‡]; Buckland, Michael, E., PhD, FRCPA[*,†,§]; O'Toole, Sandra, A., PhD, FRCPA[†,‖,¶]

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doi: 10.1097/PAP.0000000000000194
Review Articles

The Journal of Pathology: Clinical Research
J Path: Clin Res April 2017; **3**: 73–99
Published online 3 January 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/cjp2.64

Review Article

Fluorescence *in situ* hybridization in surgical pathology: principles and applications

Liang Cheng,[1,2]* Shaobo Zhang,[1] Lisha Wang,[3] Gregory T MacLennan[4] and Darrell D Davidson[1]

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JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/ College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, and Mitchell Dowsett