

LIFE LONG LEARNING PROGRAMME

TRANSVERSAL PROGRAMME

KA3 – ICT-Multilateral projects

Project title : Telepathological ASsessment of histopathological and cytological TEchniques

Project Acronym: TASTE

Project number: 519108 - LLP-2011-IT-KA3-KA3MP

Grant Agreement: 2011-4018/001-001



Pitfalls and Algorithms in Diagnostic Pathology

4th TASTE Workshop Proceedings Deliverable 7.10

Bucarest, Romania, 9th May 2014

Institutul National de Cercetare Dezvoltare in Domeniul Patologiei si Stiintelor
Biomedicale "Victor Babes"



Preface

The following presentation contains the contributions presented at the Fourth TASTE workshop, **“Pitfalls and Algorithms in Diagnostic Pathology”**, held on the 9th of May 2014 in Bucarest, Romania. The workshop has been realized in the context of the activities of the TASTE project “Telepathological ASsessment of histopathological and cytological TEchniques“, funded with the support from the European Commission.

This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

These proceedings are published on the TASTE project website www.tasteproject.eu .

Table of content

Workshop Presentations

Title of presentation	Page
<i>Workshop Introduction</i>	4
<i>The TASTE project on artefactual pitfalls</i> Emanuela Ovcin, COREP, Turin, Italy	5
<i>Diagnostic algorithms and pitfalls in defining tumour entities</i> Maria Comanescu, Victor Babes National Institute of Pathology, Bucharest, Romania	13
<i>HER2 analysis using the tricolour BDISH method: a single Institution experience on 300 cases</i> Tibor Tot, Falun Hospital, Sweden	32
<i>Pitfalls and artefacts in the diagnosis of breast cancer</i> Isabella Castellano, University of Turin, Turin, Italy	73
<i>Pitfalls in immuno-histochemical procedures</i> Gianni Bussolati, University of Turin-COREP, Turin, Italy	93
<i>Pitfalls and algorithms in diagnostic effusion cytology</i> Francesco Feoli, Institut Jules Bordet, Bruxelles, Belgium	127

Workshop Introduction

This workshop is designed for all professionals involved in manufacturing and assessing histological, cytological, immuno-histological and molecular techniques used in routine diagnostic pathology and cytology. Pathologists, pathology residents, histo-technicians, cyto-technicians, medical students and student technicians are welcome to participate. This meeting will provide information regarding the TASTE project which is part of the European Commission's Lifelong learning project. It is devoted to set technical standards in pathology. Technical pitfalls constitute a potential risk of erroneous diagnoses and their recognition, as a prerequisite for standardization and optimization of histo- and cyto-pathological preparations, is indeed the goal of the TASTE Project. Digital pathology allows open circulation of virtual slides throughout many countries of the world. It will be used in the TASTE project. The goals of discussion, training and technical standardization will be achieved by collecting top quality, average quality and artifact-damaged preparations and archiving them in a digital web based archive. The images will thereafter be standardized through user assessment sessions and made available for the interested professionals all over the world.

This fourth TASTE workshop will focus on the importance of the recognition of technical pitfalls in different areas of Pathology while, on the other side, the adoption of correct algorithmic pathways would allow to avoid misinterpretations and to reach correct diagnoses.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

TASTE

Telepathological ASessment of histopathological and cytological TEchniques



The TASTE project on artefactual pitfalls

Emanuela Ovcin

COREP

Fourth TASTE Workshop, Bucarest, 9 May 2014

With the support of the Lifelong Learning Programme of the European Union



Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

The TASTE Background



- Histological and cytological preparations are not standardized and their **quality** level is variable.
- This can affect diagnoses
- Artefactual pitfalls can lead to wrong interpretation/results



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

The TASTE project goals

How the TASTE project tackles these problems:

- enhancing (at European level) **knowledge and recognition of artefacts**
- **improving the quality** of histological and cytological preparations
- generating an **innovative training** for more reproducible diagnoses.

The TASTE results

The TASTE project gets its objectives through:

- creating the **TASTE System** with a **repository of high resolution microscopic images**
- identifying **targets** and their **needs**
- creating the **TASTE Virtual Community**



Lifelong Learning Programme



What can you do with TASTE?

- Training with **self assessment exercise** showing artefacts
- **Consulting good cases and bad ones** (artefacts) in the Library/Encyclopaedia
- **Submitting via Web**, the microscopic images of their own preparations to a **panel of internationally-recognized experts** who will give comments and suggestions.

TASTE system

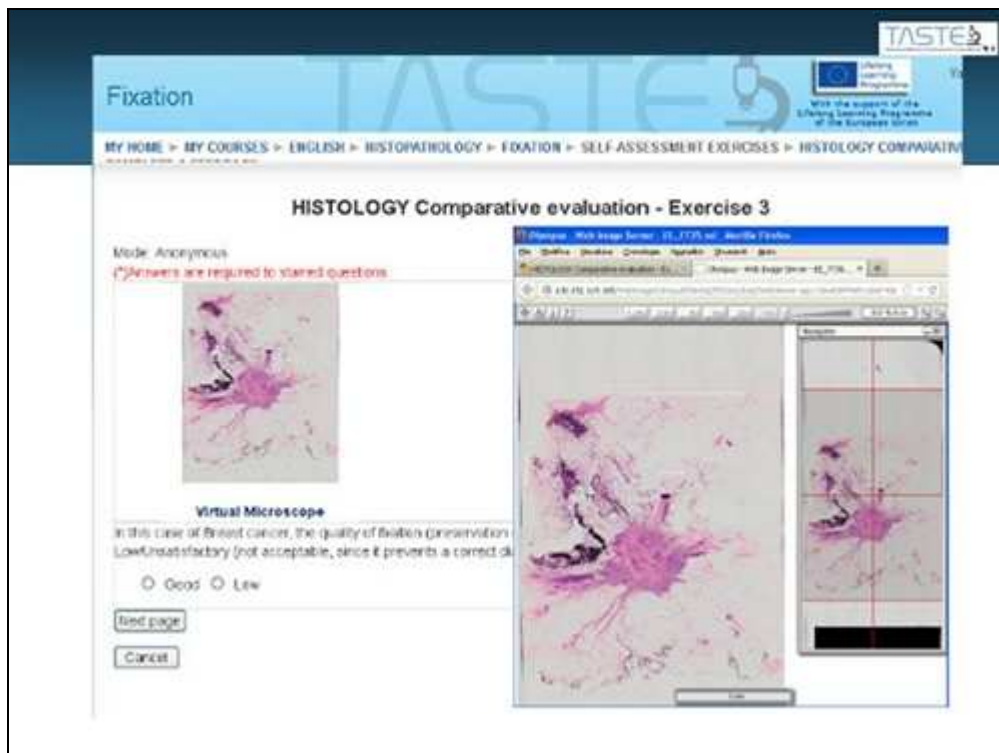
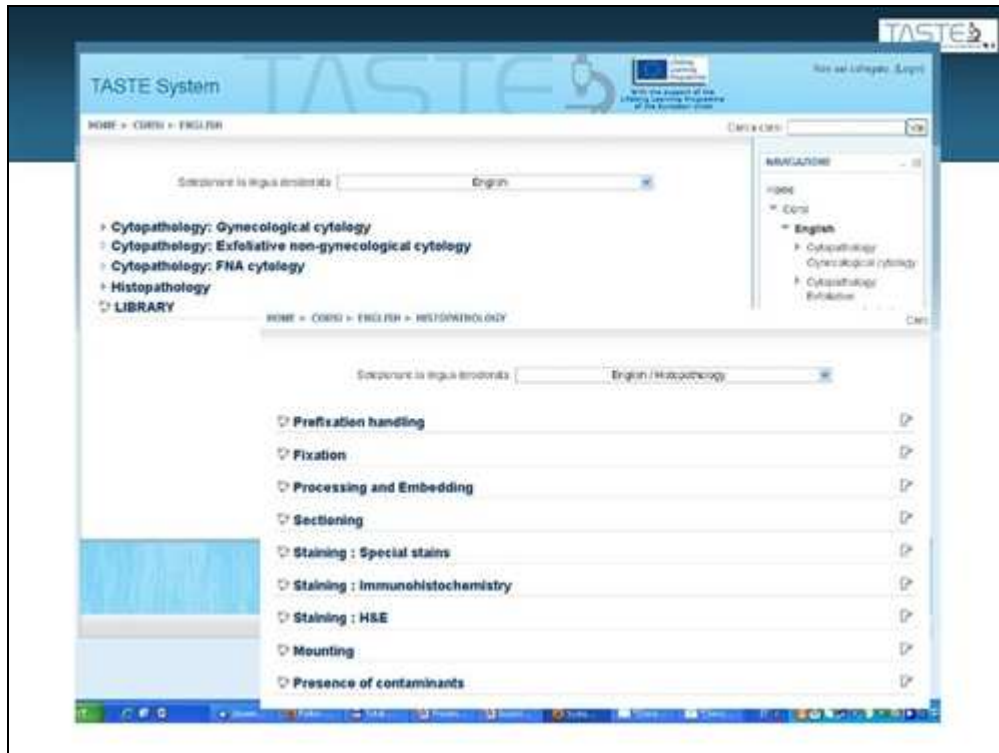
Select a language

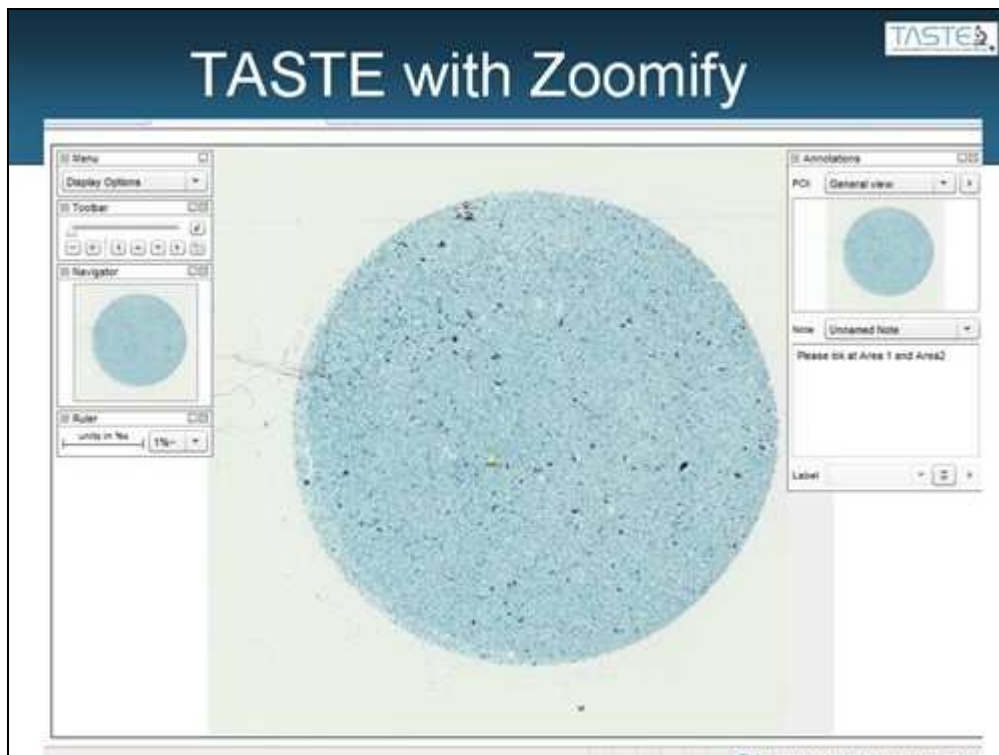
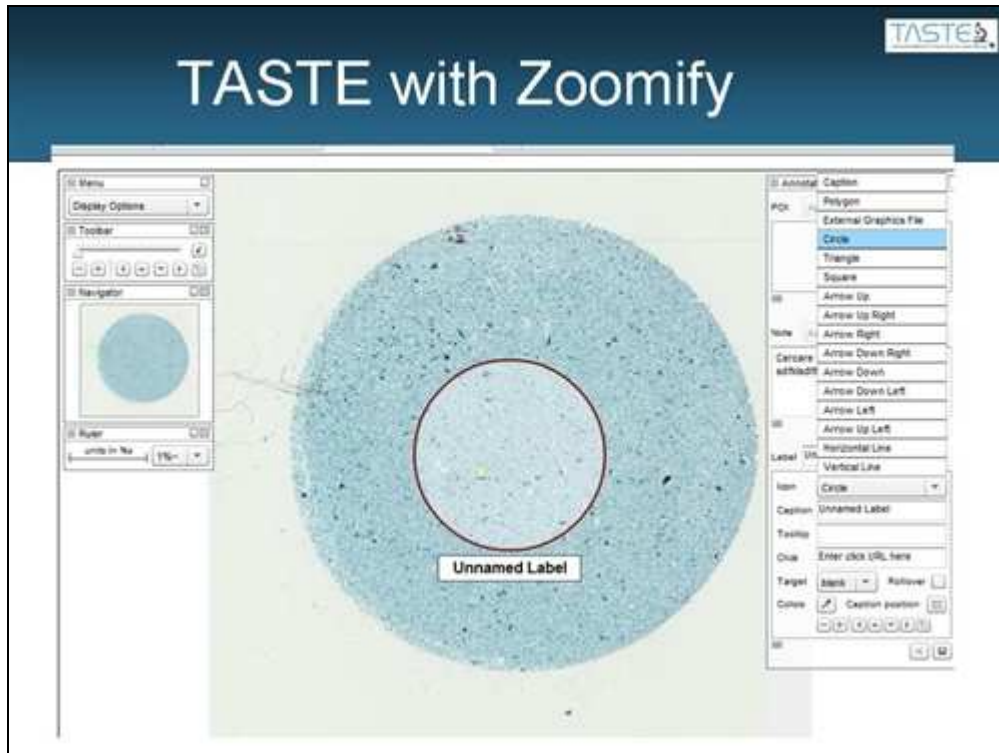
With the support of the Lifelong Learning Programme of the European Union

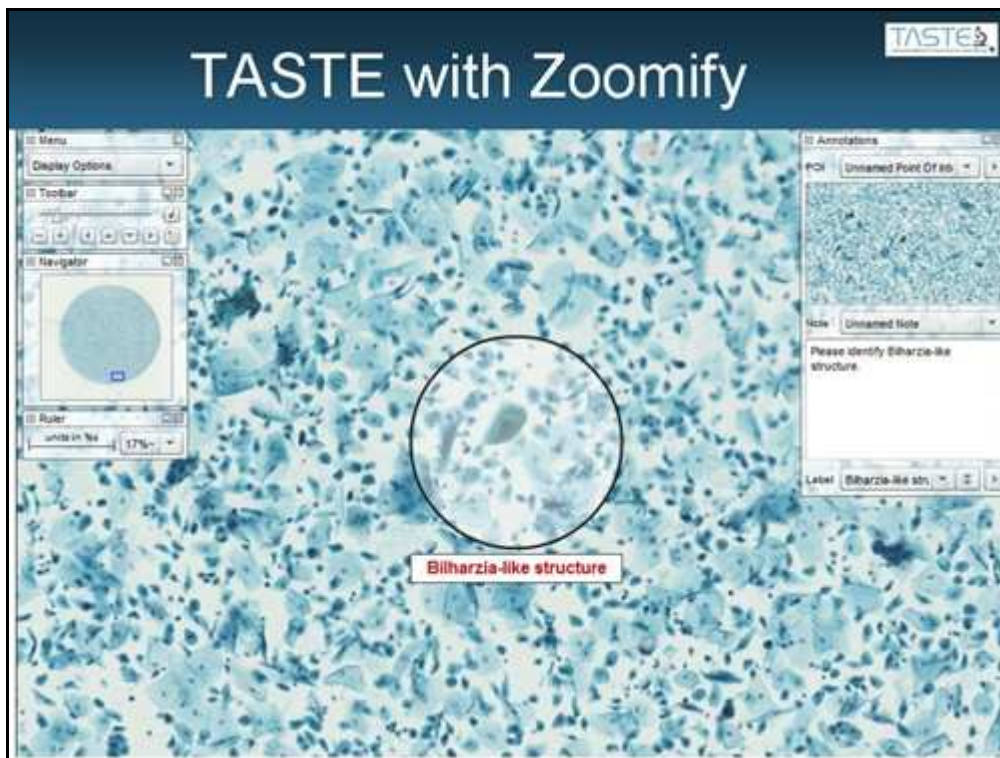
COREP SPATIMUP LmD

This project has been funded with support from the European Commission. This publication [communication] reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.

The TASTE system is available in **6 languages!**
English, Italian, French, Portuguese, Romanian, Swedish.









Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

The TASTE numbers



- **157 exercises** already available:
 - Cytopathology: Gynecological Cytology: 96
 - Exfoliative non-Gynecological Cytology: 6
 - Cytopathology: FNA cytology: 10
 - Histopathology: 45
- **2926 visits** to the Taste web site (April 2014)
- **200 external users** already involved
- **4 International TASTE Workshop**
(Falun, Bruxelles, Porto)

The TASTE targets



The TASTE project refers to:

- **pathologists,**
 - **residents in Anatomic Pathology,**
 - **technicians**
 - **students**
- from different countries.

Testing phase foreseen with all "interested parties"
for May/June: **ask here to freely participate!**



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

The TASTE Virtual Community

It is expected that the present approach will fuel a Web-based **TASTE Virtual community**.

The Need Analysis phase involved already about **200 people!**

Join the Virtual Community system for free!
Fill the subscription form here and give it back to us or write to infotaste@corep.it

TASTE References & Contacts

- TASTE project web site:
www.tasteproject.eu
- TASTE system:
www.system.tasteproject.eu
- To join the Virtual Community,
get a free login or further info:
infotaste@corep.it

Thanks for your
attention!



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Diagnostic algorithms and pitfalls in defining tumour entities.

Approach to Pathologic Diagnosis
of Uterine Cancer



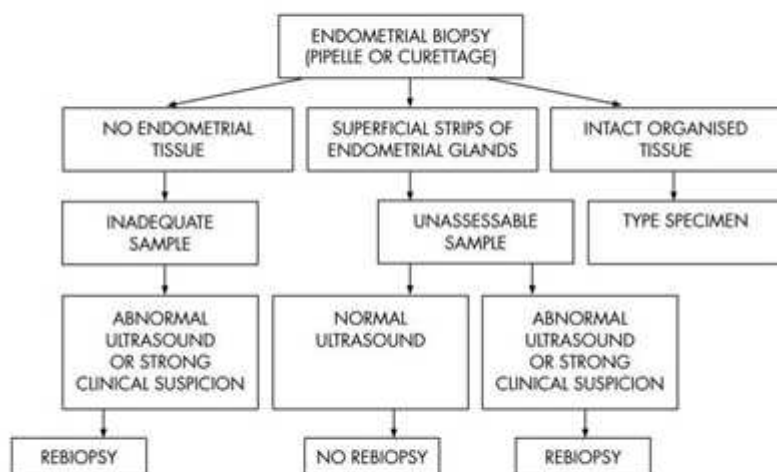
How useful is IHC in gynecological pathology? ~10%

- Positive diagnosis: rare
- Differential: sometimes
- Molecular targets, prognostic factors:
unconclusive





- Dilatation & curettage
- Pipelle



J Clin Pathol, Aug
2006; 59(8): 801-812



Biomarkers

- **Epithelial**

- CK: AE1/AE3, KL1, MNF116, CK 7
- EMA

- **Mesenchimal**

- Vimentin
- Desmine, Caldesmon, CD10 (CALLA), WT1, Act –
celule stromale

Others:

p53, ER/PGR (ADK endometrioid), PTEN (-),



Endometrial tumors

- **Polyp**
- **Endometrial hyperplasia**
- Non-atypical hyperplasias
 - Simple hyperplasia
 - Complex hyperplasia without atypia
- Atypical hyperplasias
 - Simple atypical hyperplasia
 - Complex atypical hyperplasia
- **Endometrial cancer**





Epithelial

- Endometrioid adenocarcinoma
- Mucinous adenocarcinoma
- Serous adenocarcinoma
- Clear cell adenocarcinoma
- Mixed carcinoma
- Squamous cell carcinoma
- Transitional cell carcinoma
- Small cell carcinoma
- Undifferentiated carcinoma

Stromal

- Endometrial stromal sarcoma; low grade
- Endometrial stromal nodule
- Undifferentiated stroma sarcoma

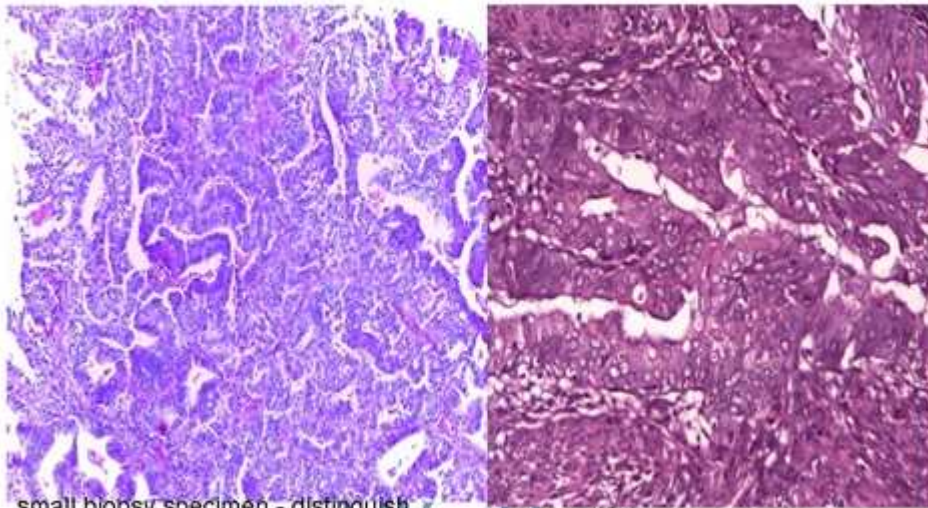


7

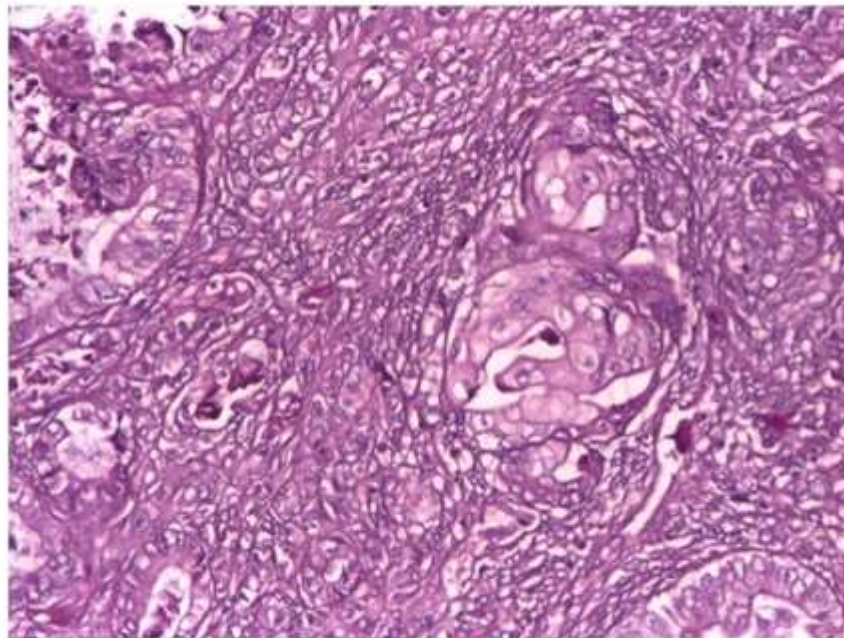
Endometrioid Adenocarcinoma

- Architecture resembles endometrial glands: tubuloglandular growth
- Cells resemble endometrial glands: columnar cells.
- Nuclear pleomorphism/atypical mitoses restricted to highest end of grade 3.
- Squamous differentiation is common.
- *WHO criteria for squamous differentiation* (any one criteria is sufficient)
 - Keratinization
 - Intracellular bridges
 - Constellation of 3 or more of these:
 - Sheet-like growth without glands or palisading
 - Sharp cell margins
 - Eosinophilic and glassy cytoplasm
 - Decreased N/C ratio compared to glandular component





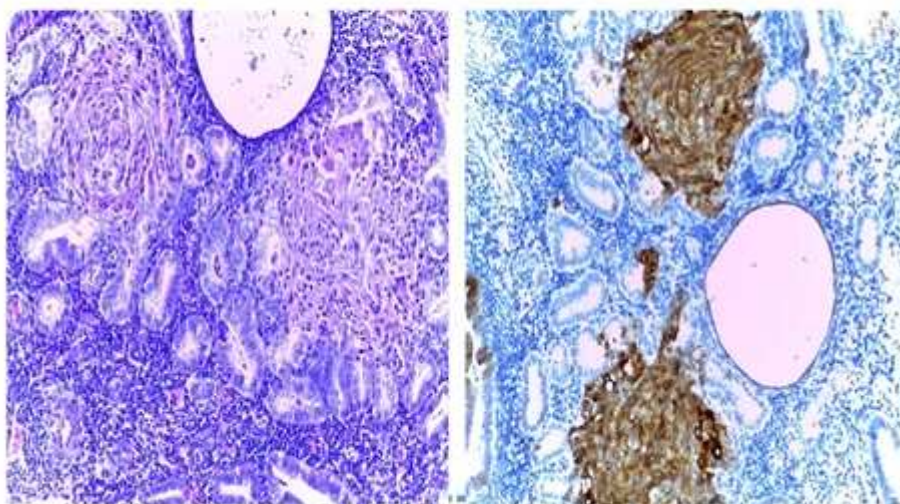
small biopsy specimen - distinguish
between an atypical hyperplasia and a
grade 1 endometrioid adenocarcinoma

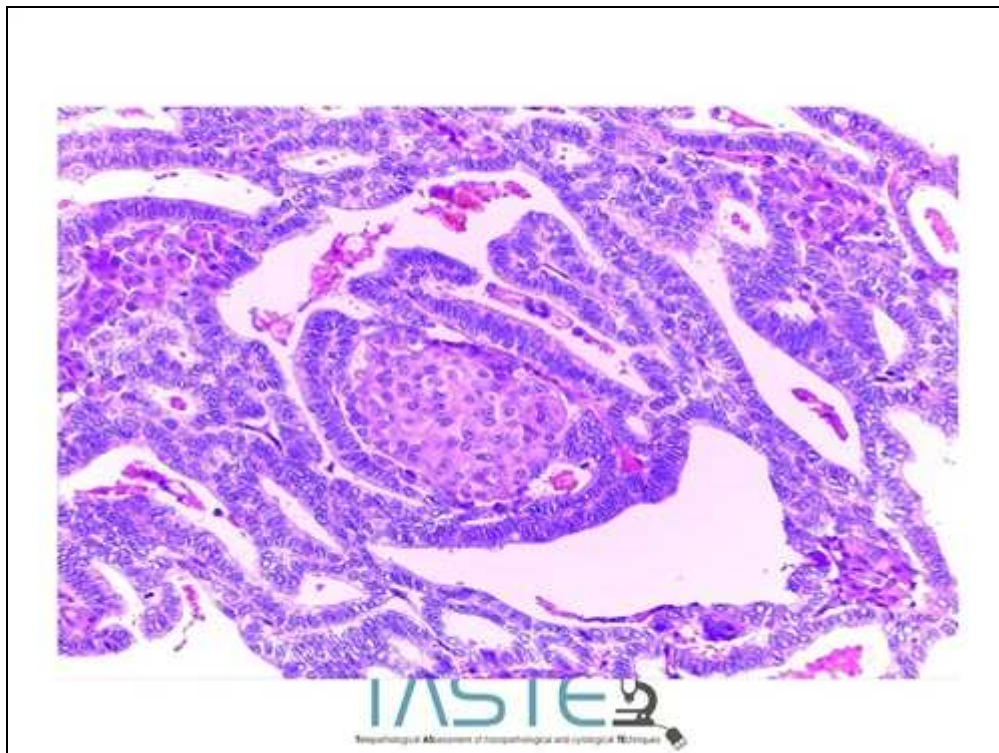
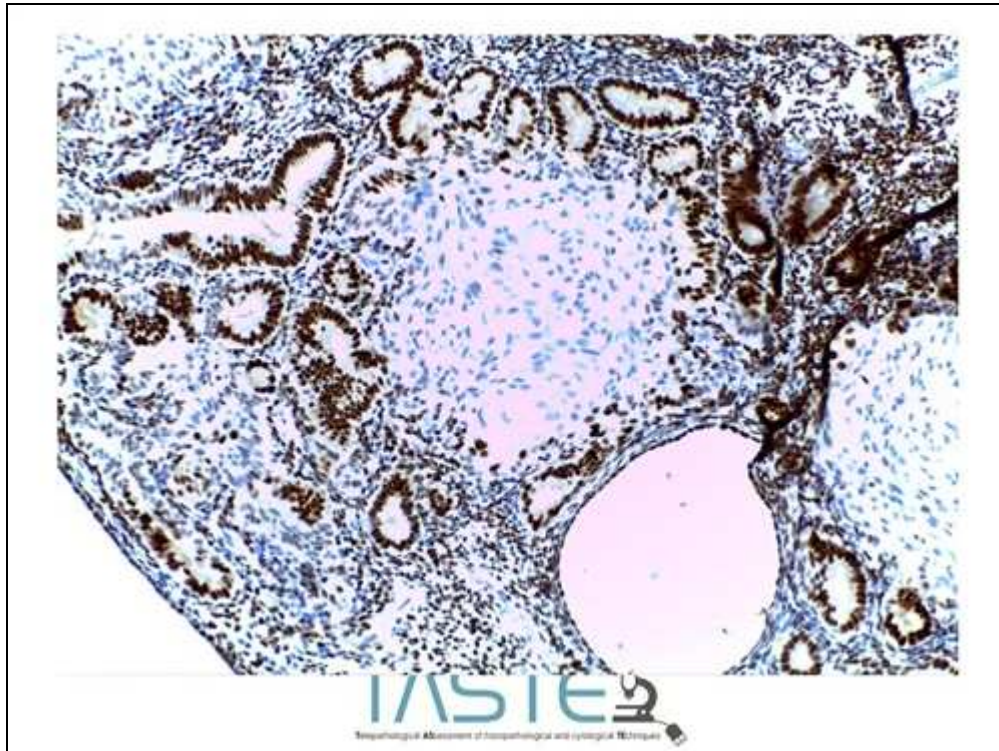


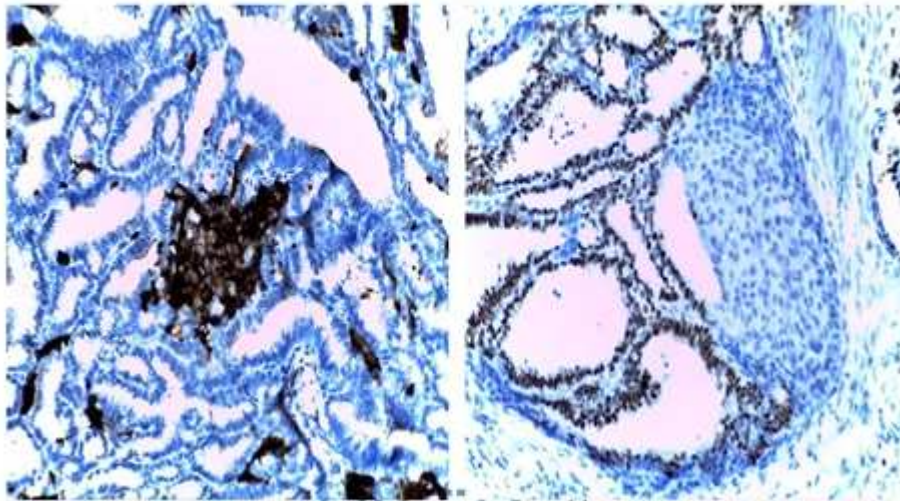


Epithelial metaplasias

- squamous or mucinous
- coexist with hyperplasia / carcinoma.
- when there is florid squamous metaplasia of the keratinising or morular type
- the squamous elements can be so extensive that the underlying glandular component is almost totally obliterated.







CD10

TASTE
Transpathological Assessment of transpathological and cytological Techniques

Panel - ADK

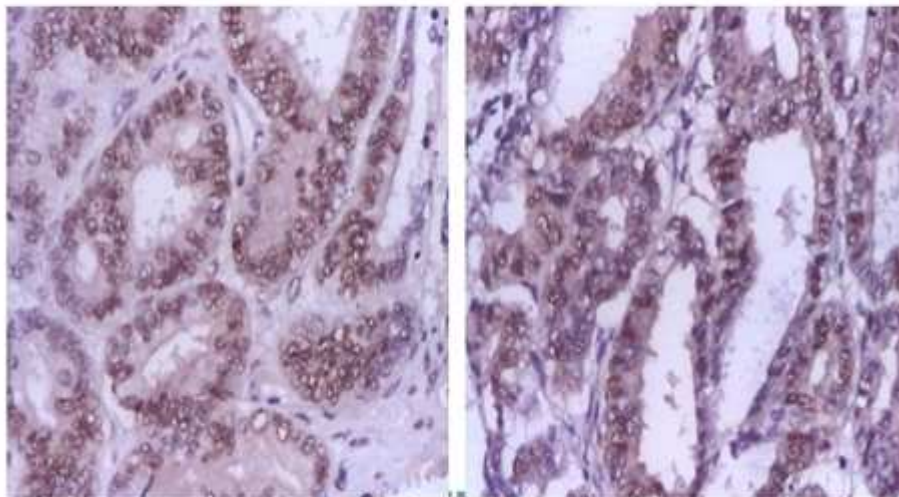
- ER, PGR
- p53
- EGFR
- C-erbB-2
- EMA
- CK7
- CA125
- CD44, β -catenin
- Ki67
- PAX2

TASTE
Transpathological Assessment of transpathological and cytological Techniques



ER, PR

- Variable positivity in endometroid adenocarcinomas
- Negative / weakly positive areas in clear cell carcinomas and uterine serous carcinomas
- zonal weak positivity / negative in uterine poorly differentiated carcinomas
- Intense positive reaction - favorable prognosis





p53

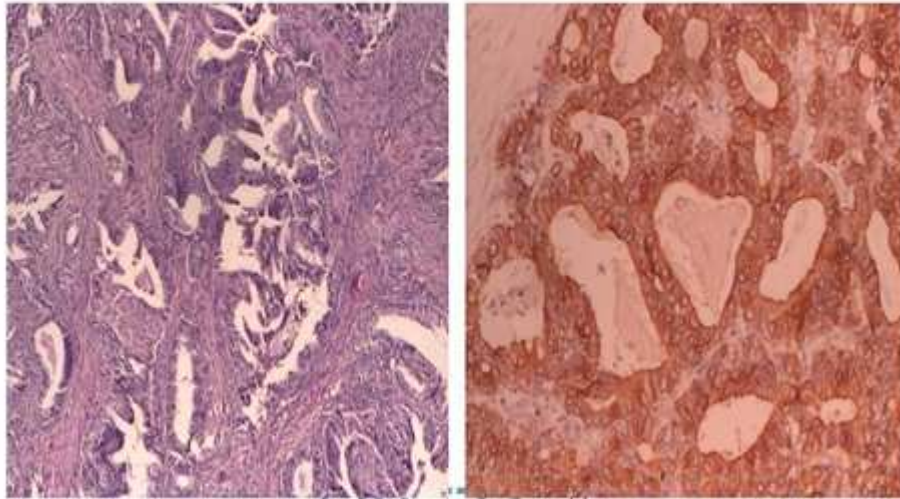
- Intense and diffuse positive reaction in uterine serous carcinomas
- Weak positive reaction, zonal / negative in well differentiated endometrioid adenocarcinomas
- Intense positive reaction in poorly differentiated adenocarcinomas and leiomyosarcomas
- Intense positive reaction - poor prognosis



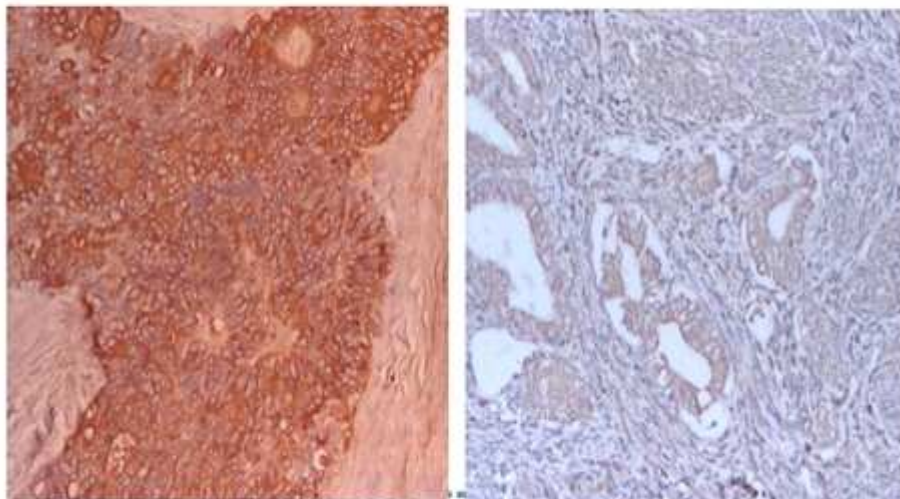
No pathognomonic positive marker of “endometrioid” differentiation

- Immunophenotype: CK, EMA, vimentin
 - areas of squamous differentiation CK34beta E12 positive, CEA negative
- DD:
 - endocervical and ovarian ADK: CK, EMA, CEA
 - RCC, thyroid, salivary gland, mezoteliomul: expresie imunofenotipica dubla
- prognostic factors
 - HP: type and grade, myometrial invasion, peritoneum, lymph nodes :
 - IHC ER / PgR (good), p53, Ki67 (bad); cerbB2, VEGF (uncertain)

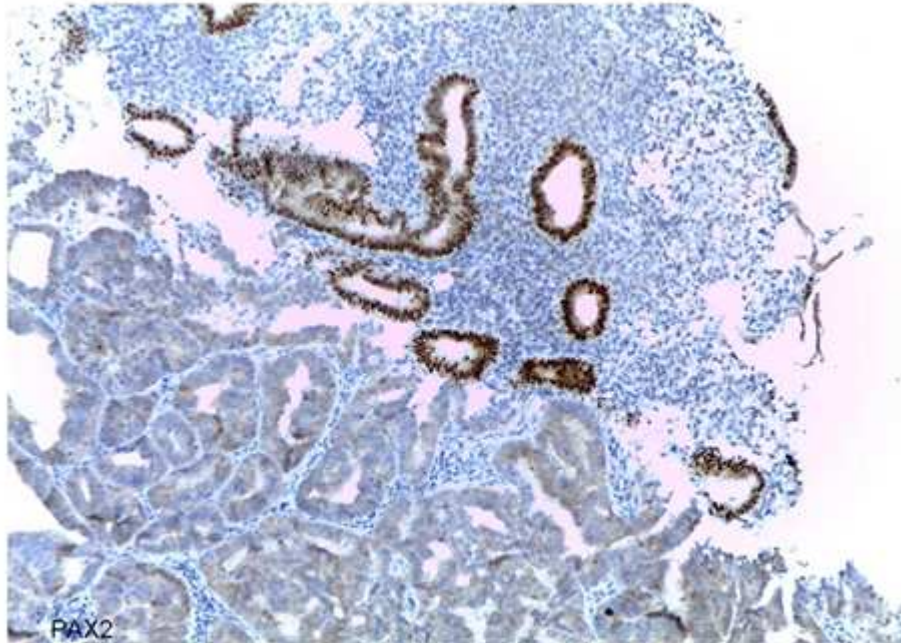




TASTE
Transpathological Assessment of transpathological and cytological Techniques



TASTE
Transpathological Assessment of transpathological and cytological Techniques

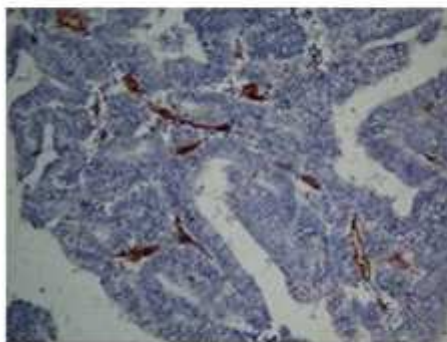
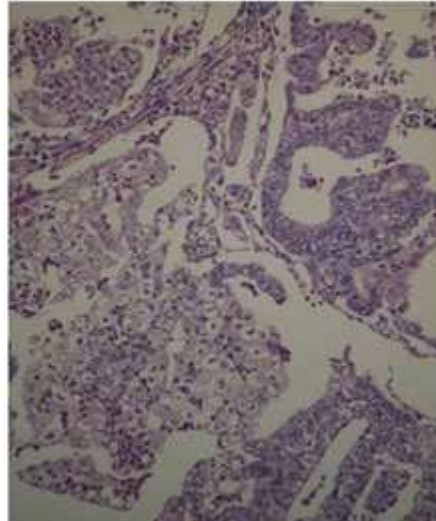
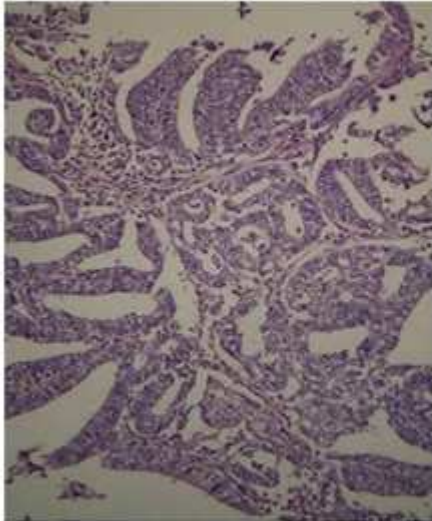


TASTE
Transpathological Assessment of transpathological and cytological Techniques

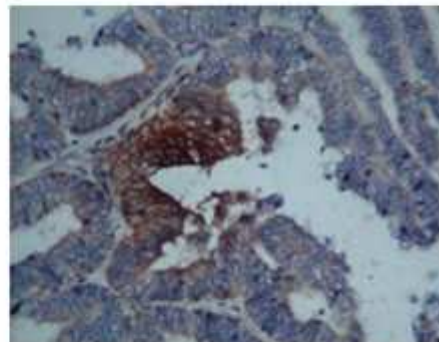
Serous Carcinoma

- Architecture often mixed: papillary, micropapillary, semi-solid with slit-like branching spaces
- Pleomorphic, high grade tumor cells; irregular/polygonal shape, rarely columnar shape.
- High nuclear: cytoplasmic ratio, minimal cytoplasm, nuclear enlargement, coarse chromatin, macro-nucleoli
- Atypical mitoses

TASTE
Transpathological Assessment of transpathological and cytological Techniques



EGFR-20x



Cerb2-20x





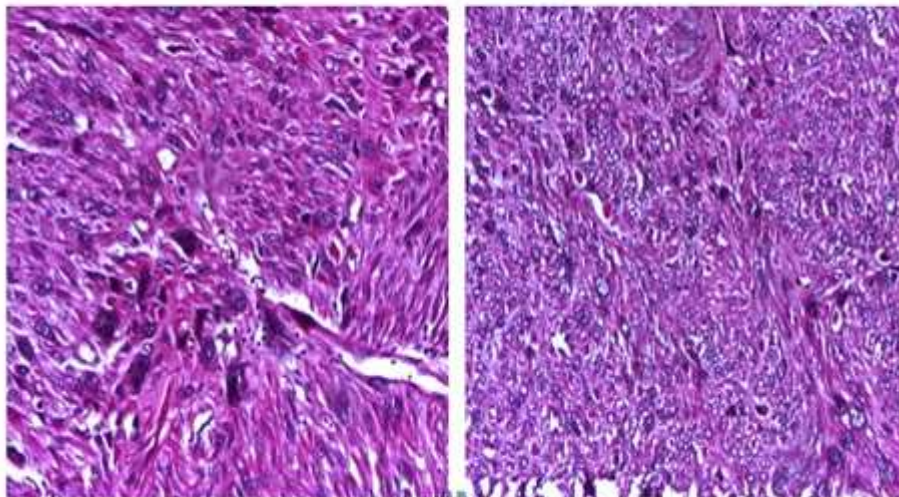
Education and Culture DG

Lifelong Learning Programme



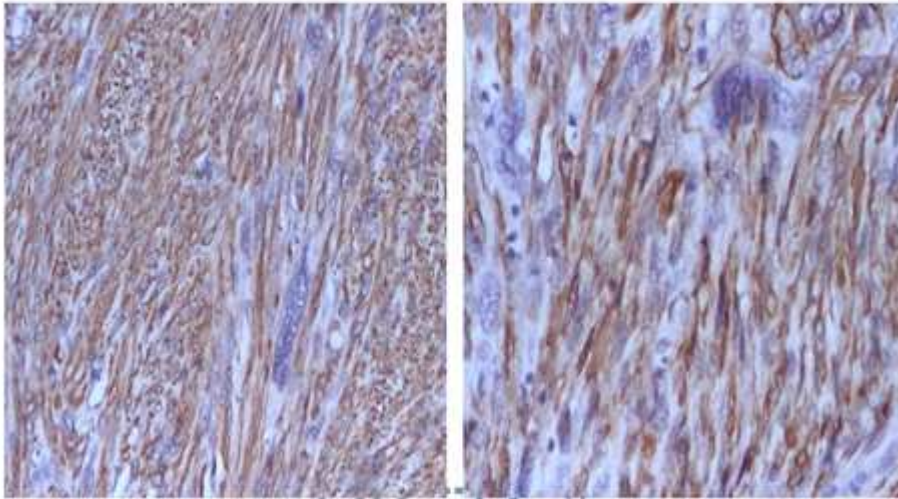
Education, Audiovisual & Culture
Executive Agency

- Endometrial stromal tumors and uterine smooth muscle tumors represent two major types of uterine mesenchymal tumors.
- differentiate a highly cellular leiomyoma - benign clinical course- from an ESS - Aggressive

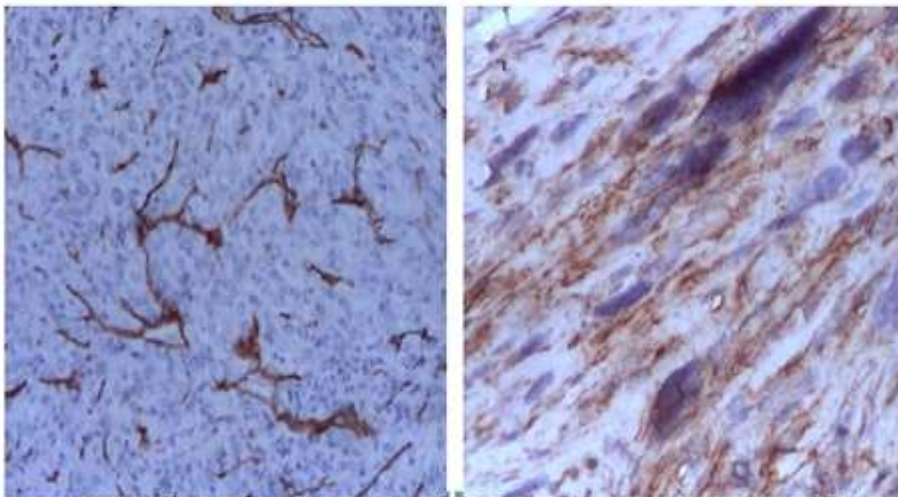




Actin



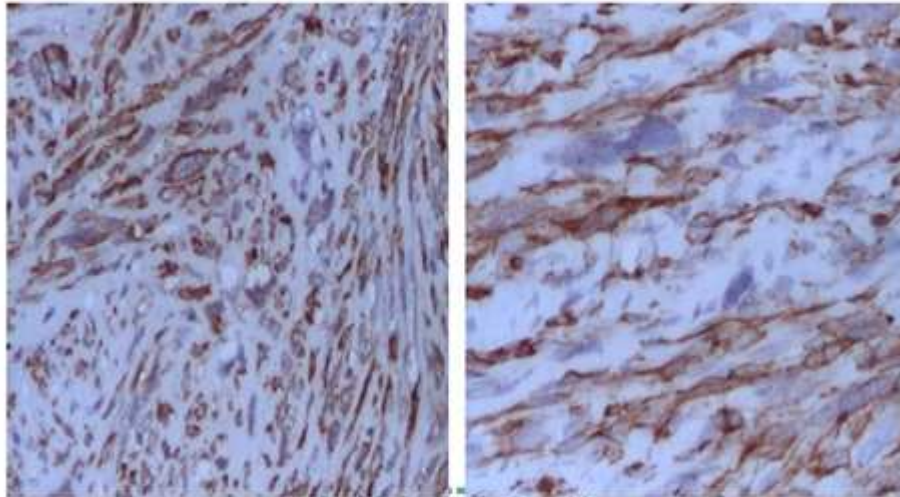
TASTE
Transpathological Assessment of histopathological and cytological Techniques



CD34

CD44

TASTE
Transpathological Assessment of histopathological and cytological Techniques

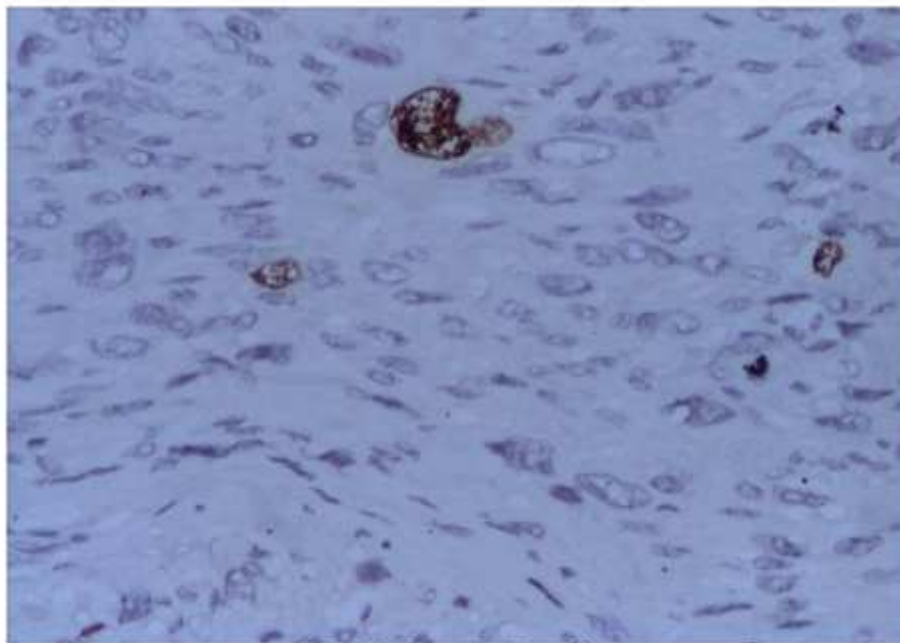


Caldesmon

Desmin



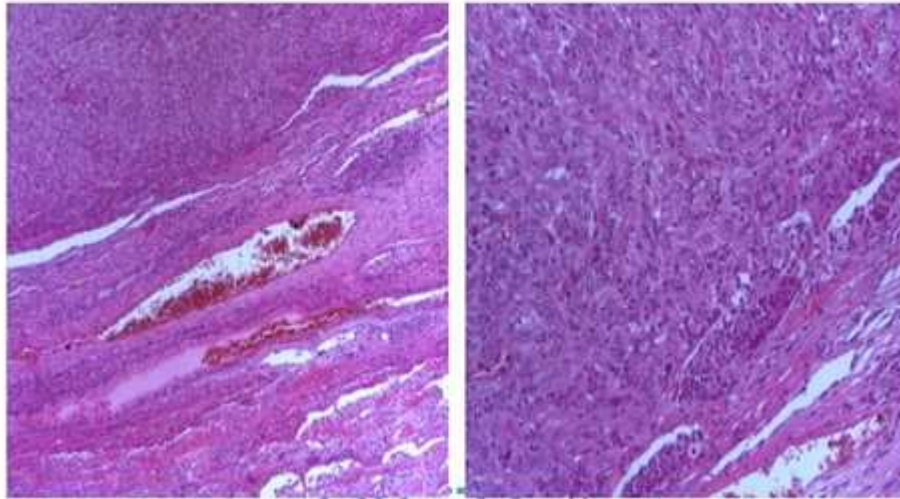
Transpathological Assessment of transpathological and cytological Techniques



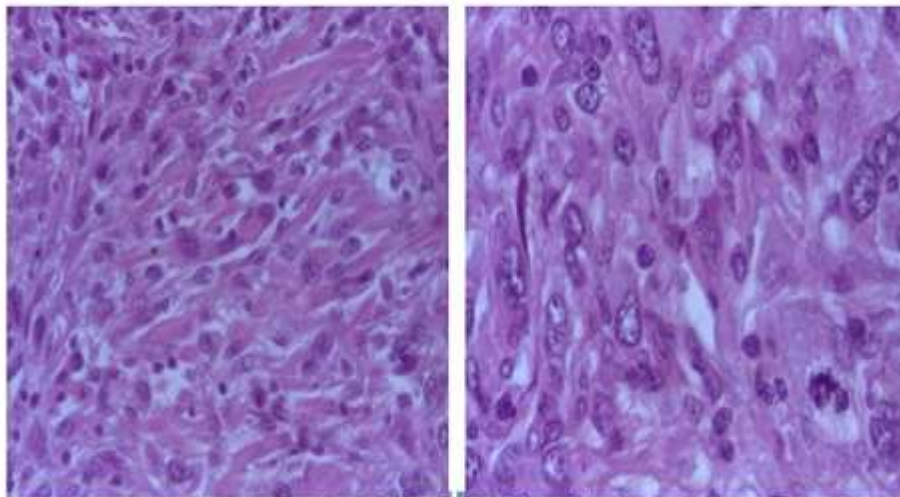
ki67



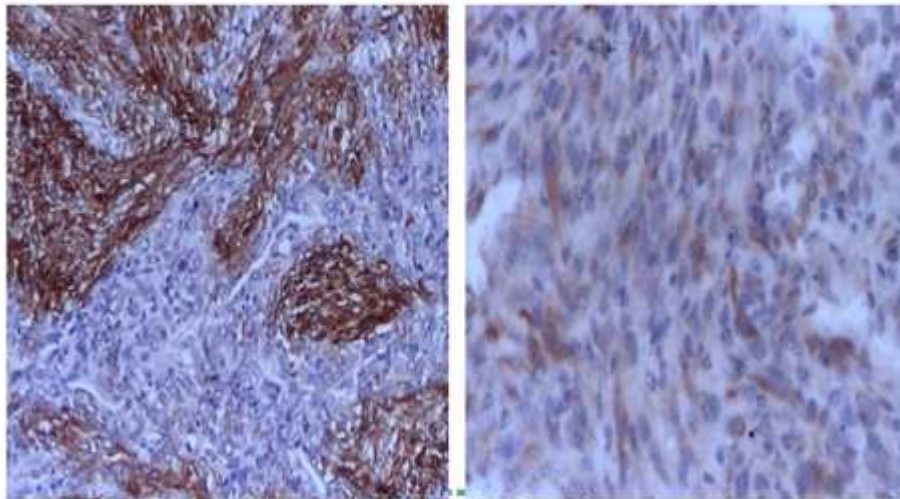
Transpathological Assessment of transpathological and cytological Techniques



TASTE
Transpathological Assessment of transpathological and cytological Techniques



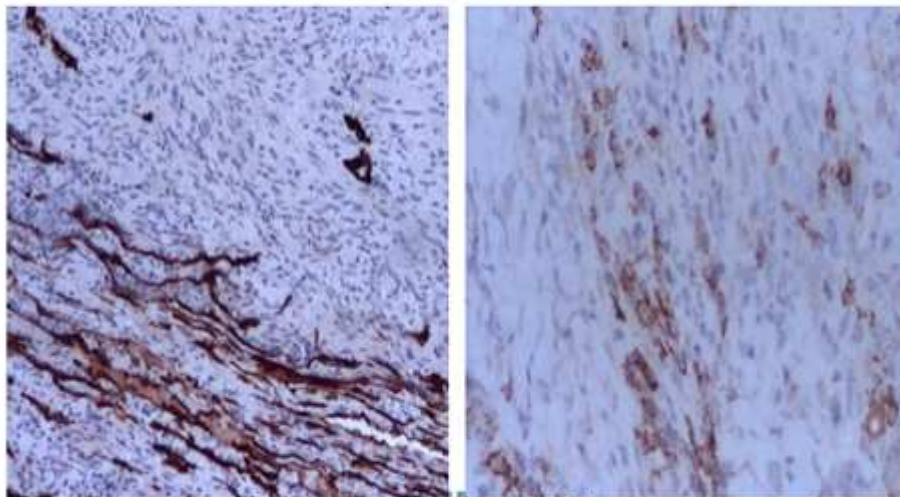
TASTE
Transpathological Assessment of transpathological and cytological Techniques



Actin

Desmin

TASTE
Transpathological Assessment of transpathological and cytological Techniques



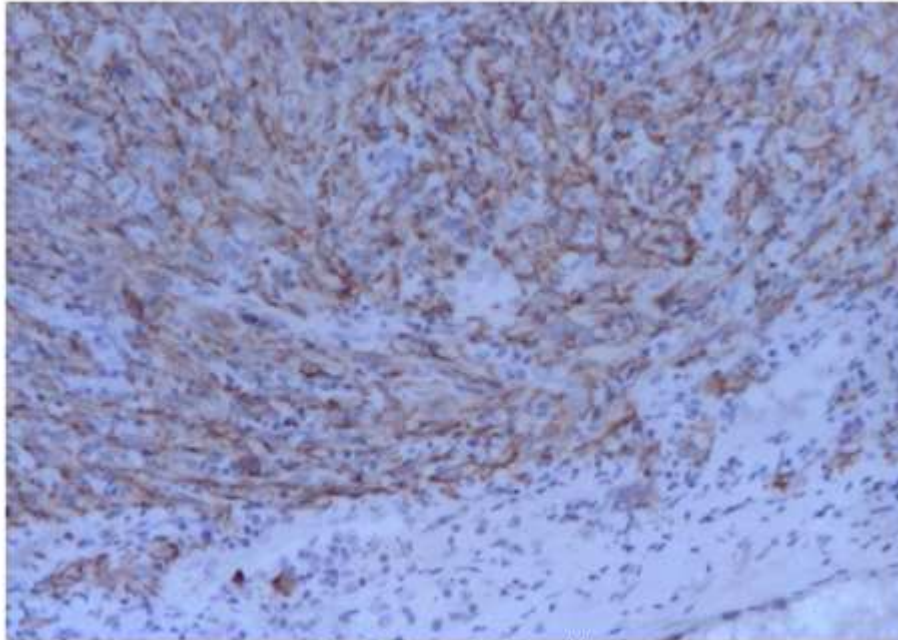
AE1/AE3

EMA

TASTE
Transpathological Assessment of transpathological and cytological Techniques



Lifelong Learning Programme



CD10





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

TASTE Workshop, Bucharest, Romania, 2014

**HER2 assessment with tricolor BDISH method:
a single institution experience on 300 cases**

Tibor Tot

Uppsala University

Falun Central Hospital, Sweden





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Manifesto on Optimal Pathology



EBCC Council

timely way.

Note: about the optimal breast cancer pathology manifesto

This manifesto was prepared by a European Breast Cancer (EBC) Council working group and launched at the European Breast Cancer Conference in Glasgow on 20 March 2014.

It sets out optimal technical and organisational requirements for a breast cancer pathology service, in the light of concerns about variability and lack of patient-centred focus.

It is not a guideline about how pathology services should be performed. It is a call for all in the cancer community – pathologists, oncologists, patient advocates, health administrators and policymakers – to check that services are available that serve the needs of patients in a high quality,

EBC Council welcomes all feedback on this first version, which is a working document that will be edited to produce a final version, and will also inform a more detailed position paper.

Please email Davi.Kaur@ecco-org.eu with suggestions.

The members of the working group are:

- Alberto Costa (scientific director, European School of Oncology, Milan, Italy)
- Emiel Rutgers (head of surgery, The Netherlands Cancer Institute)
- Tibor Tot, associate professor of pathology, Uppsala University, and head of laboratory medicine at Central Hospital Falun, Dalarna, Sweden
- Giuseppe Viale (director, department of pathology, European Institute of Oncology, Milan, Italy)

Breast cancer pathology - a manifesto for optimal care



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Prognostic parameters

Radiological/surgical

- Distant metastasis
- Lymph node status
- Tumor size
- Multifocality
- Extent
- Resection margins

Oncological

- Histology grade
- ER/PR status
- HER-2 status
- Proliferation
- Molecular subtypes

Breast cancer pathology - a manifesto for optimal care

This manifesto calls for:

High quality, timely breast cancer pathology services that meet the needs of cancer patients and oncologists

By:

- Ensuring a full range of diagnostic and prognostic pathology services is available
- Promoting professional development for pathologists and their technicians, and full integration with multidisciplinary working in cancer care
- Organising hospital pathology services to deliver timely information for decision making and ensuring that pathologists are part of the team visible to patients
- Monitoring and reporting undue variability in pathology results nationally and internationally
- Introducing quality standards and reporting that are focused on services and care for breast cancer patients
- Promoting pathology as a vital and major discipline with substantial career potential in clinical practice and translational research



Part 2: Optimal breast cancer pathology – organisation

This section of the manifesto lists optimal organisational factors for pathology from individual to national level.

Individuals – professional expertise and development requirements

- A high level of competency in all aspects of breast pathology, including both classical morphological and modern molecular/genetic aspects, according to current guidelines
- Continuous education and improvement of diagnostic skills
- Understanding of the clinical consequences of every detail in the pathology report
- Networking with experts for discussing professional views

Departmental requirements

- Access to all relevant clinical information for each patient
- Full attention to breast specimens and a team of technicians that provides high quality breast preparations
- Day-to-day multidisciplinary teamworking, such as with detailed correlation between radiology and pathology, and with molecular biology and pathology
- Understanding of the capabilities and limitations of imaging methods, biopsy modalities and histopathology techniques
- Pathologists should be part of the patient-facing team and available to explain reports to patients
- At least, an informal system for obtaining or giving second opinions on selected cases, involving experts at external institutions

Hospital requirements

- High quality of work, checked with continuous internal monitoring to meet international standards
- Adherence to Good Laboratory Practice (GLP) guidelines and participation in external quality control schemes
- Monitoring of multidisciplinary teamwork among departments, not only in centres with oncology specialists but also in general hospitals
- External laboratories when used should also follow guidelines and be subject to quality control
- Services should be available in a timely way to every breast cancer patient: unnecessary wait times should be identified and eliminated
- Maintenance of a high-quality biobank for research

Health system requirements

- A commitment to a pathology service that is organised to meet the needs of multidisciplinary teams and their patients
- A national pathology quality standards organisation
- A monitoring system to audit key breast cancer pathology parameters across cancer centres and hospitals
- National and international auditing of laboratory testing
- A strategy for referring borderline cases to experts for second opinions
- An education and training strategy to ensure that sufficient pathologists and technicians are in post
- Incorporating patient-advocate viewpoints in service reviews



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Next steps

- Final publication of this first version of the manifesto, and a position paper, to follow
- Circulation to breast cancer advocacy groups
- Support from major European societies concerned with oncology (ECCO, ESP, ESMO, ESSO, ESTRO etc)
- Lobbying of European Parliament/national governments

Manifesto on Optimal Pathology



Standardization of assessment

- Guidelines
- Registry
- Population-based data comparison
- External quality control

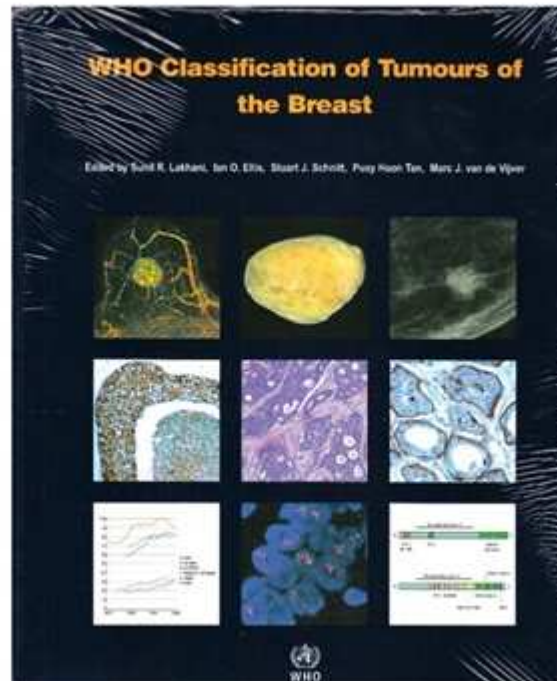


Breast pathology, Sweden, 2014

- WHO blue book
- AJCC Staging handbook: TNM7
- CAP guidelines
- EWGBCSP – European guidelines
- Socialstyrelsens nationella riktlinjer för bröstcancersjukvård 2007 (revised 2014)
- KVAST – Swedish pathology guidelines
- Nationell vårdprogram
- Regionala (RCC) vårdprogram
- Ackrediterade labs metodbeskrivningar

Breast pathology, Sweden, 2014

- **Oncology guidelines**
(ASCO, San Gallen, San Antonio)
- **Svenska Bröstcancergruppen**
- **Regionala Cancercentra**



European guidelines for quality assurance in breast cancer screening and diagnosis

Author: European Commission - Directorate-General for Health and Consumer Protection



Price

Publisher

Publication date 01 January 2007

ISBN

Publication synopsis The fourth edition of this bestseller has been developed with input from over 200 professionals from 23 countries. New issues include digital mammography and advice for specialist breast units.



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

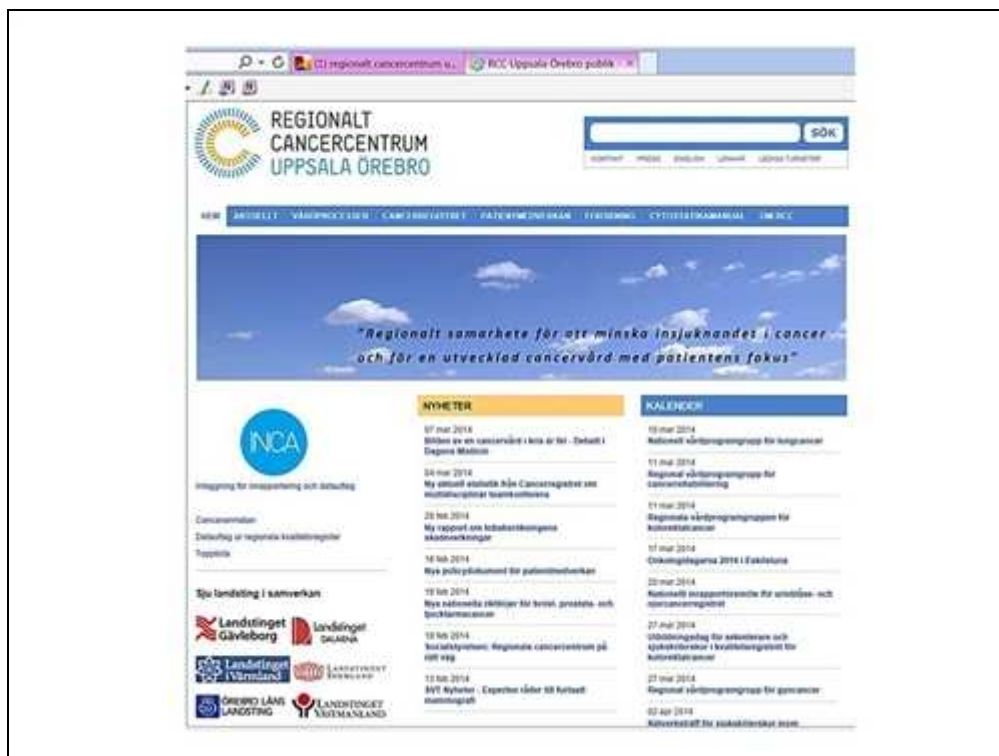




Table 2. Histopathologic Features Suggestive of Possible HER2 Test Discordance	
Criteria to Consider ¹	
New HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:	
Histologic grade 1 carcinoma of the following types:	
Infiltrating ductal or lobular carcinoma, ER and PgR positive	
Tubular (at least 90% pure)	
Mucinous (at least 90% pure)	
Cribriform (at least 90% pure)	
Adenoid cystic carcinoma (90% pure) and often triple negative	
Similarly, a new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:	
Histologic grade 1 carcinoma of the following types:	
Infiltrating ductal or lobular carcinoma, ER and PgR positive	
Tubular (at least 90% pure)	
Mucinous (at least 90% pure)	
Cribriform (at least 90% pure)	
Adenoid cystic carcinoma (90% pure) and often triple negative	
If the initial HER2 test result in a core-needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed:	
Tumor is grade 3	
Amount of invasive tumor in the core biopsy is small	
Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core	
Core biopsy result is equivocal for HER2 after testing by both ISH and IHC	
There is doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error	
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; PgR, progesterone receptor.	
¹ Criteria to consider if there are concerns regarding discordance with apparent histopathologic findings and possible false-negative or false-positive HER2 test result.	

Published Ahead of Print on October 1, 2013; DOI: 10.1200/JCO.2012.42.9899

Journal of Clinical Oncology

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

ASCO-CAP 2013

- Not only newly diagnosed primary invasive carcinomas but also recurrent and metastatic disease should be tested



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

ASCO-CAP 2013

- IHC: >10% - > 30%, > 10% cut off for 3+

Rakha et al. Histopathology 2014

ASCO-CAP 2013

- Rare gland-forming tumors and micropapillary carcinomas may show incomplete membrane staining and should be 2+



Education and Culture DG

Lifelong Learning Programme



ASCO-CAP 2013

- Acceptable fixation time 6-72 h (instead of 5-48h)

ASCO-CAP 2013

Register:

- Time and duration of fixation
- Type of fixative
- Adequacy of the sample for evaluation
- Number of the observers



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Guidelines

- Too many
- Too complicated
- Too often revised

HER2 basics



Education and Culture DG

Lifelong Learning Programme



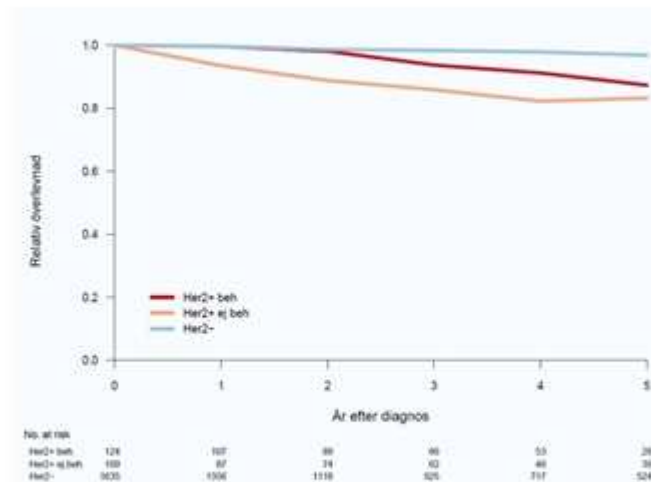
Education, Audiovisual & Culture
Executive Agency

HER2

- Human Epidermal Growth Factor Receptor Family encoded on chr 17 (17q12-21.32)
- 15-20% of breast cancer overexpress HER2 and/or exhibit gene amplification

The HER-2/neu oncogene is a member of the erbB-like oncogene family, and is related to, but distinct from, the epidermal growth factor receptor. This gene has been shown to be amplified in human breast cancer cell lines. In the current study, alterations of the gene in 189 primary human breast cancers were investigated. HER-2/neu was found to be amplified from 2- to greater than 20-fold in 30% of the tumors. Correlation of gene amplification with several disease parameters was evaluated. **Amplification of the HER-2/neu gene was a significant predictor of both overall survival and time to relapse in patients with breast cancer.** It retained its significance even when adjustments were made for other known prognostic factors. Moreover, HER-2/neu amplification had **greater prognostic value than most currently used prognostic factors, including hormonal-receptor status, in lymph node-positive disease.** These data indicate that this gene may play a role in the biologic behavior and/or pathogenesis of human breast cancer

Salmon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987 Jan 9;235(4785):177-82



Figur 1. Relativ överlevnad för bröstcancerpatienter med tumörer <10 mm uppdelat på HER2-status, diagnosår 2005-2013.

Conclusions:

- HER2 positivity is associated with more aggressive disease and decreased survival.
- Trastuzumab (monoclonal antibody targeting HER2) alone or in combination with chemotherapy is efficient in HER2+ cancer.
- HER2 testing is recommended in all breast cancers to determine potential eligibility for treatment with trastuzumab.



HER-2 status as an imprecise predictor of response to anti-HER2 therapy

- Response in 36-79% of the patients**

Bullock K, Blackwell K. Clinical efficacy of taxane-trastuzumab combination regimens for HER-2-positive metastatic breast cancer. *Oncologist* 2008;13:515-25

- Some HER2 negative patients respond**

Paik S, Kim C, Wolmark N: HER2 status and benefit from adjuvant trastuzumab in breast cancer. *N Engl J Med* 2008, 358:1409-11

FISH	Dual SISH
"Gold standard"	FDA Approved
Less optimal histology details	Optimal histology details
Takes 3 days	Takes few hours
Signals fade over time	Signals preserved for archival review
Heterogeneity less evident	Heterogeneity evident
Assessed by technicians	Assessed by pathologists

Clark HZ, Bhargava R. Bright-field Microscopy for HER2 Gene Assessment. Not Just DISH-ful Thinking? *Editorial, Am J Clin Pathol* 2013;139:137-39



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

FDA approved tests

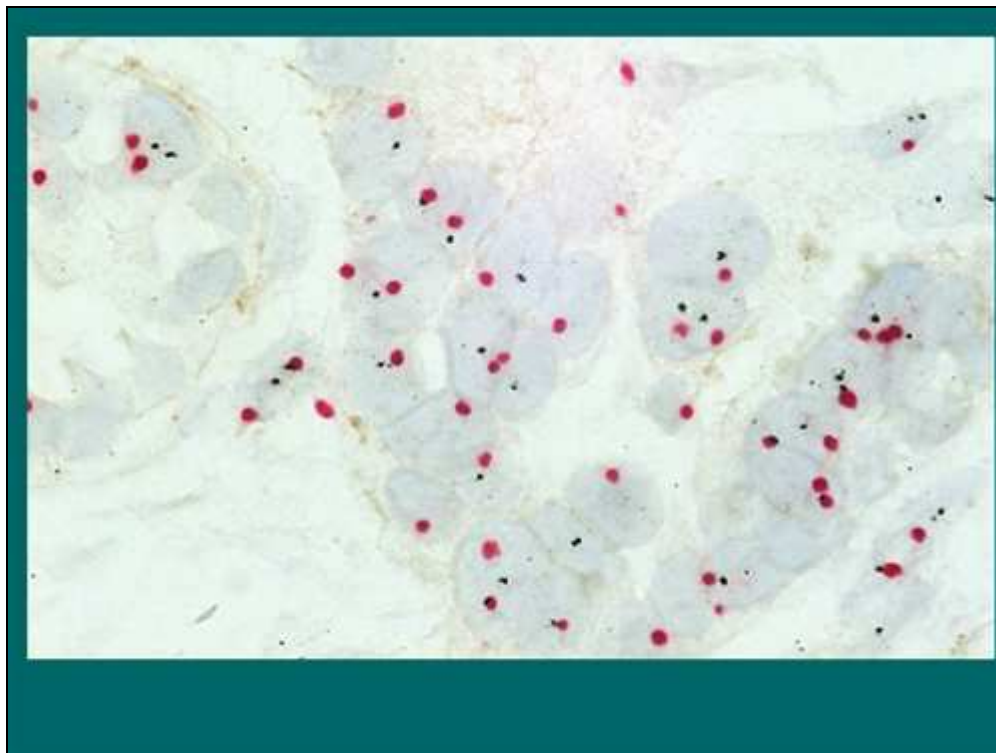
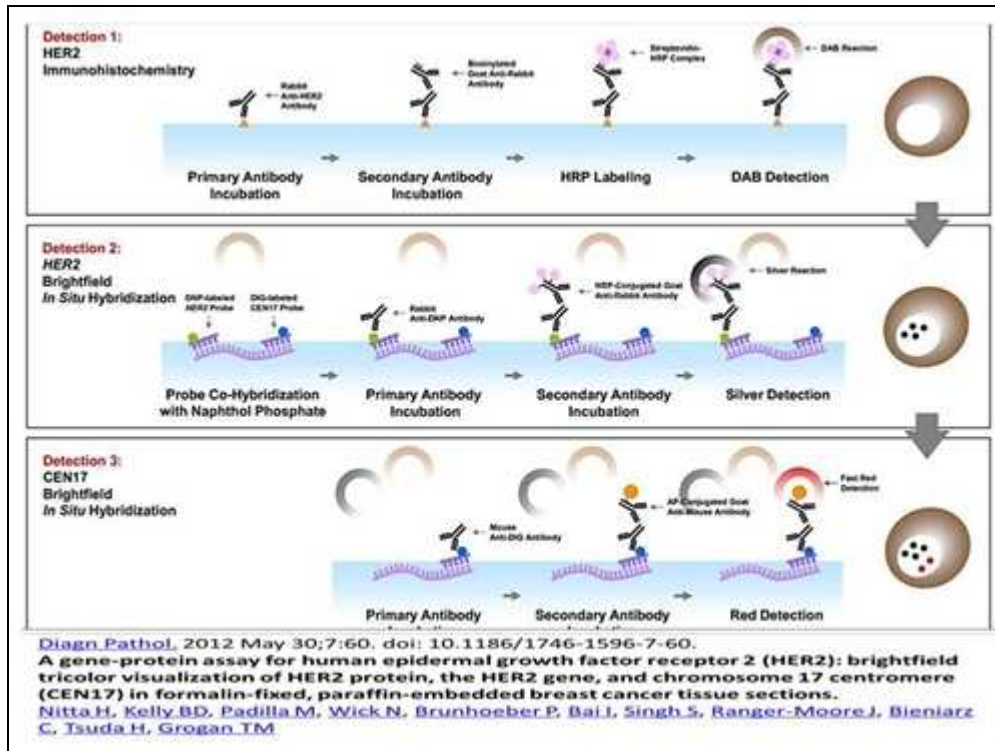
- Immunohistochemistry
- In situ hybridisation

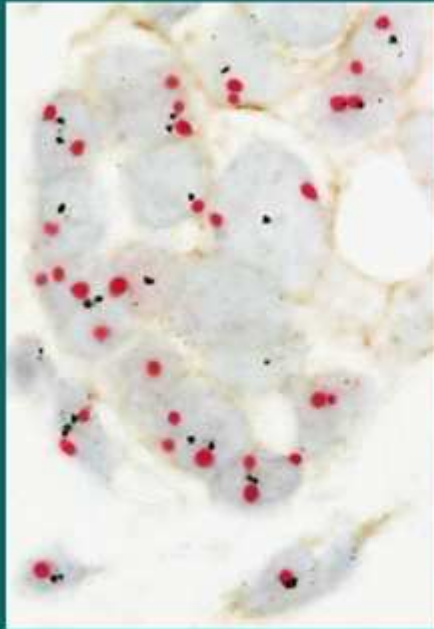
DNA probe coupled to fluorescence (FISH),
chromogenic (CISH) or silver (SISH)

Combinations: bright-field dual ISH (CISH + Sish
= BDISH or dual hapten, dual color ISH, DDISH

Gene-protein assay: tricolor (DISH+IH)

Tricolor BDISH

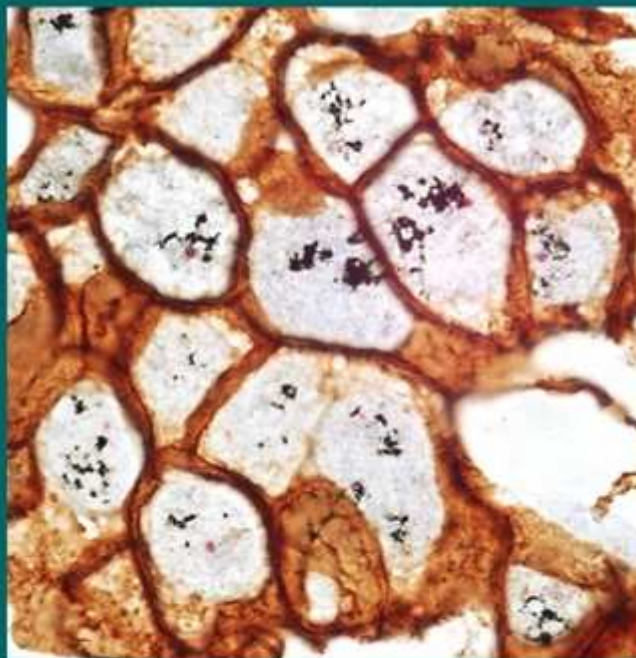




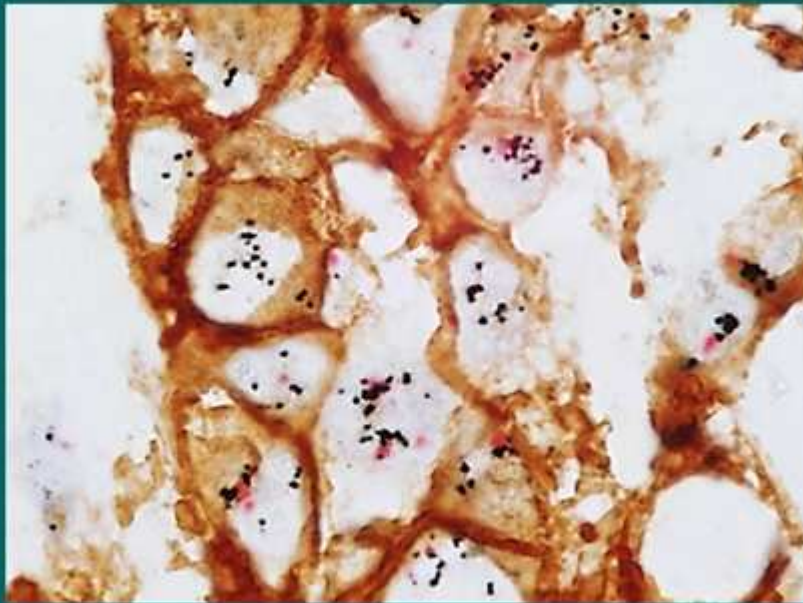
Tricolor brightfield dual in situ hybridization
HER2 non-amplified (negative)



HER2 amplified (positive)



HER-2 protein expression 3+, cluster gene amplification



HER-2 protein expression 3+, gene amplification



HER-2 protein expression 2+, gene amplification

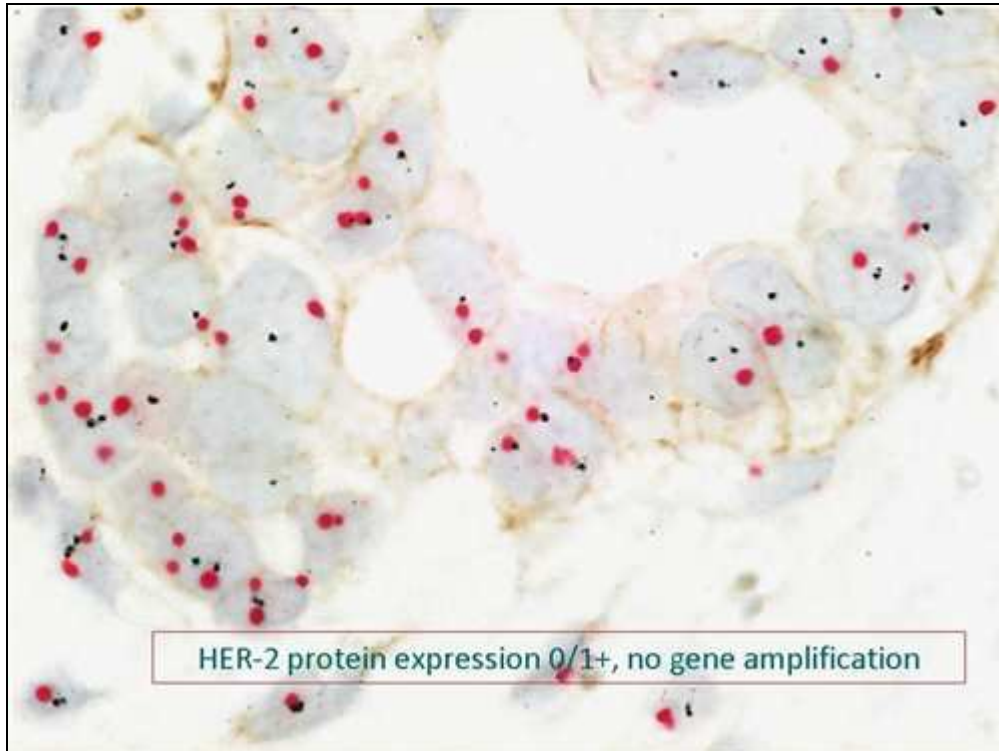


Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



BDISH compares favorable with FISH in IH2+ cases

Gao FF, Dabbs DJ, Cooper KL, Bhargava R.

Bright-Field HER2 Dual In Situ Hybridization (DISH) Assay vs Fluorescence In Situ Hybridization (FISH): Focused Study of Immunohistochemical 2+ Cases. Am J Clin Pathol. 2014 Jan;141(1):102-10. doi: 10.1309/AJCP6CXS8OSRHXIR



Education and Culture DG

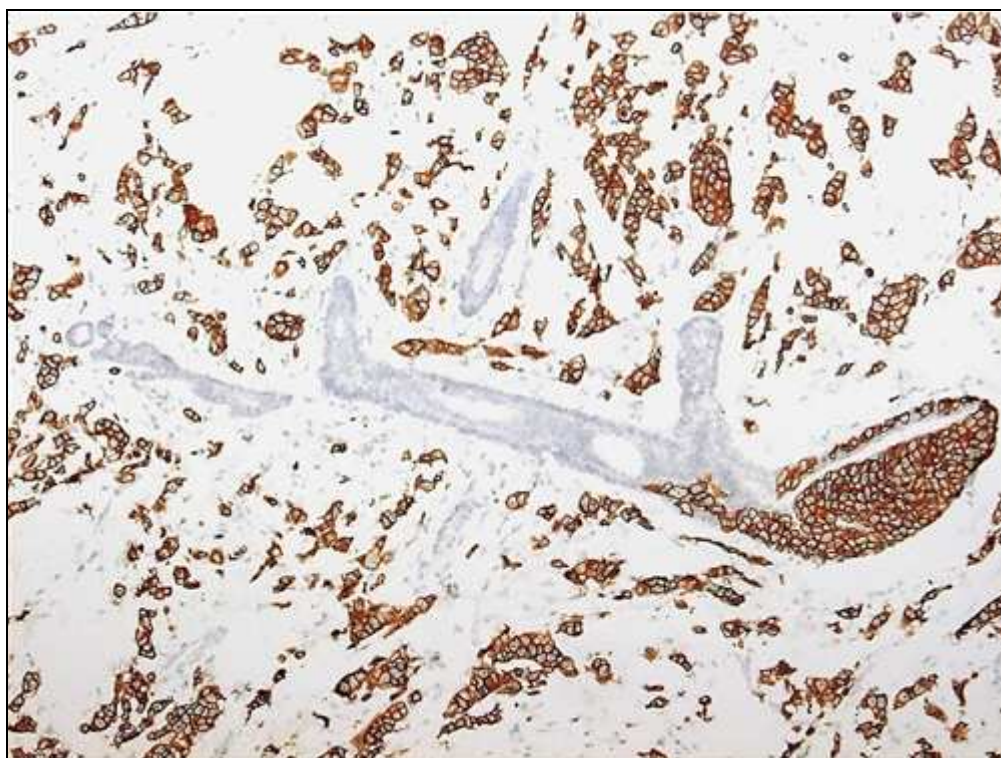
Lifelong Learning Programme

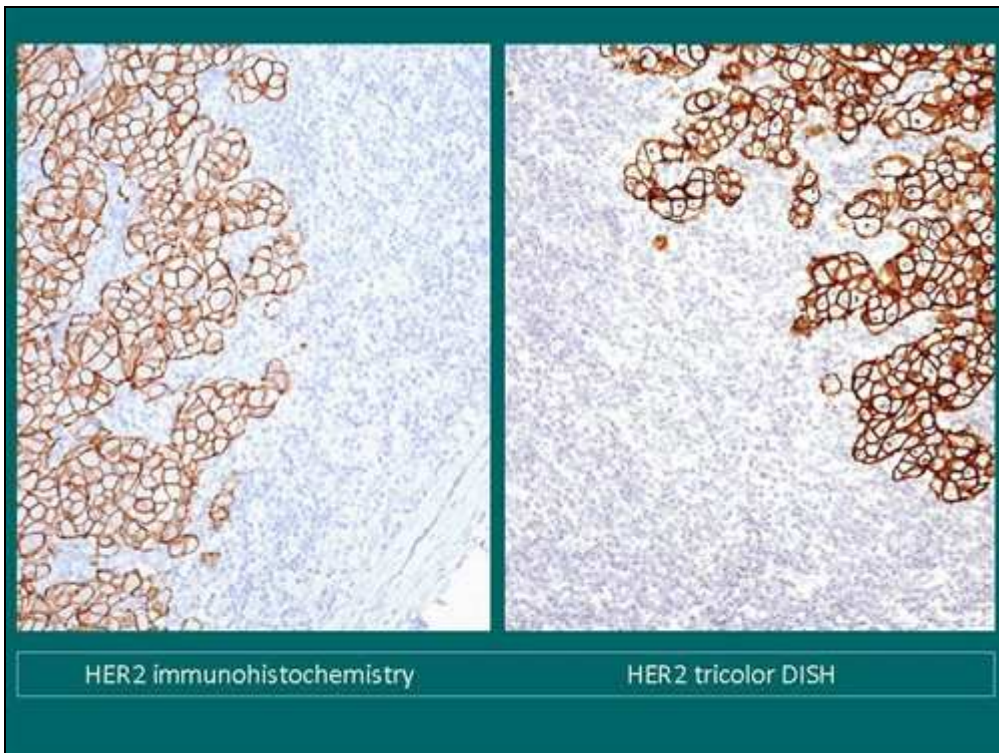
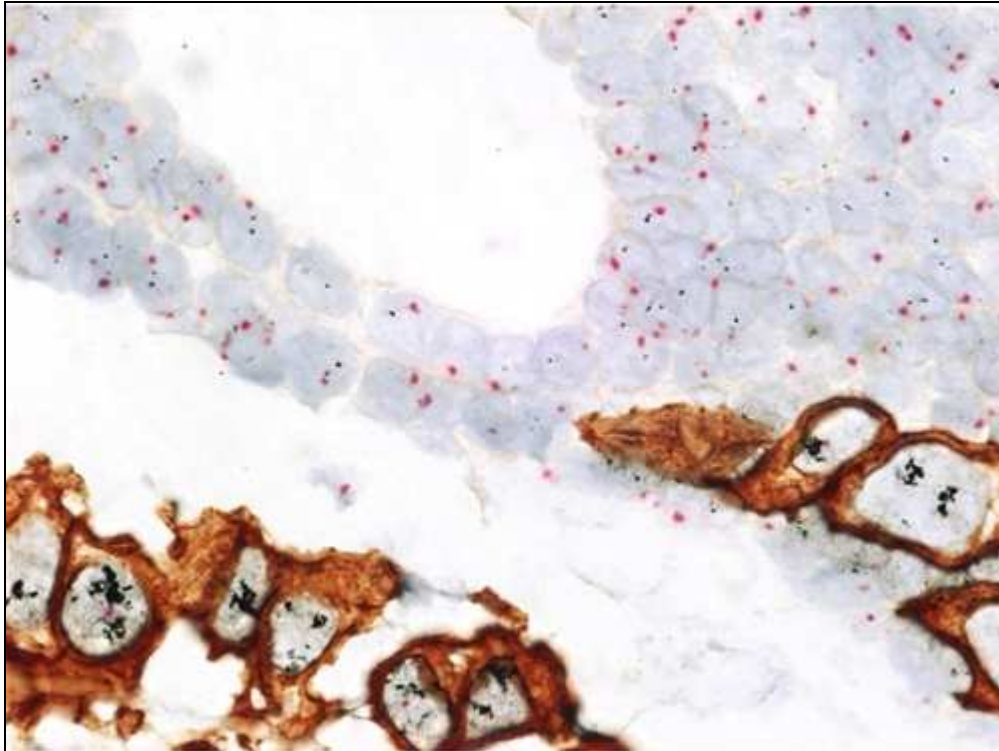


Education, Audiovisual & Culture
Executive Agency

Problems

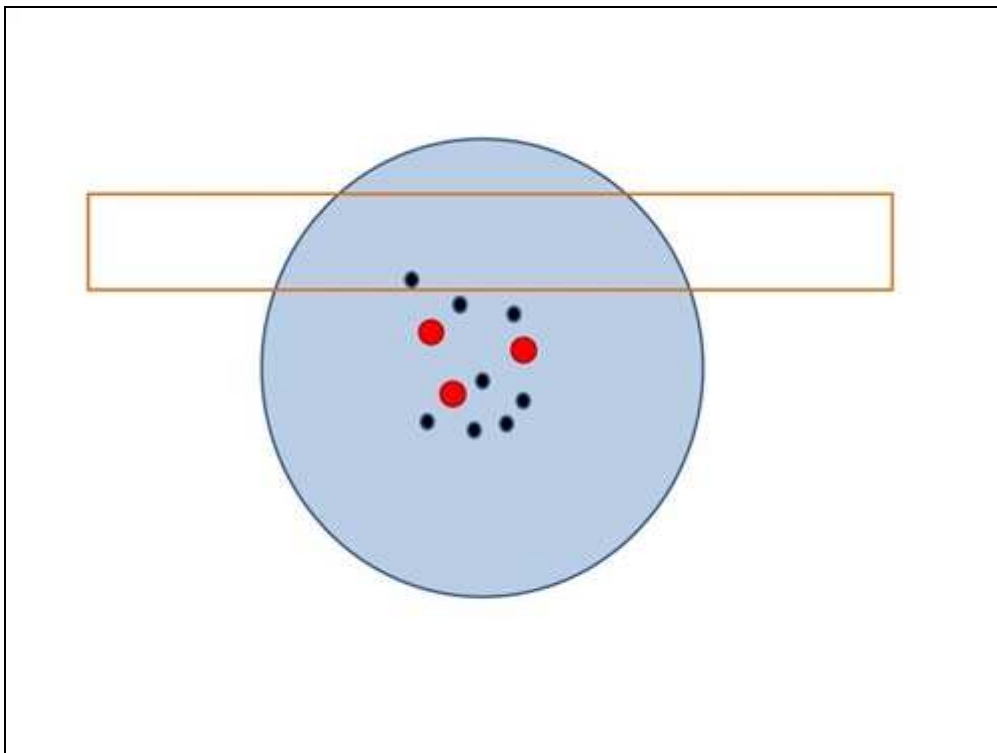
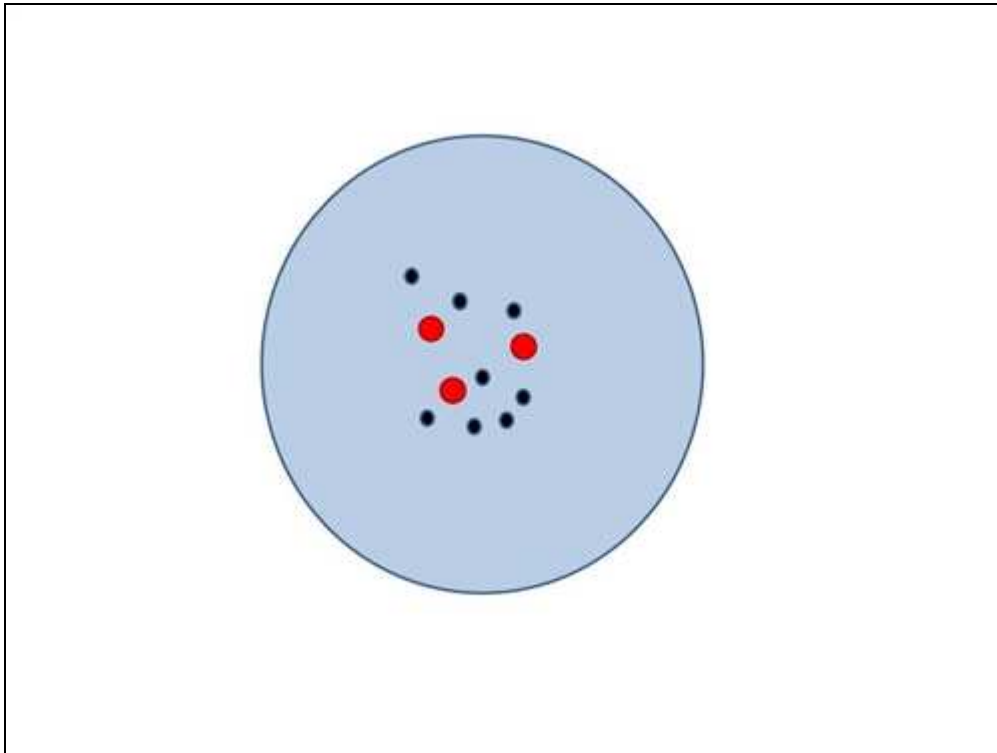
- Technical issues
- Criteria
- Interobserver/interlaboratory agreement
- heterogeneity

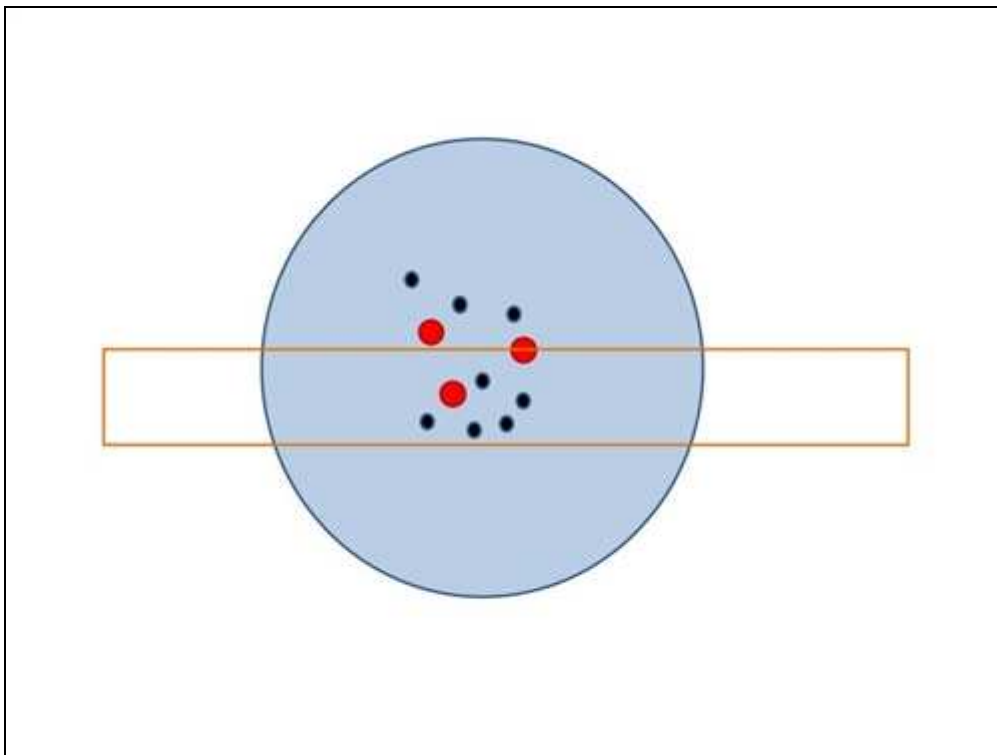
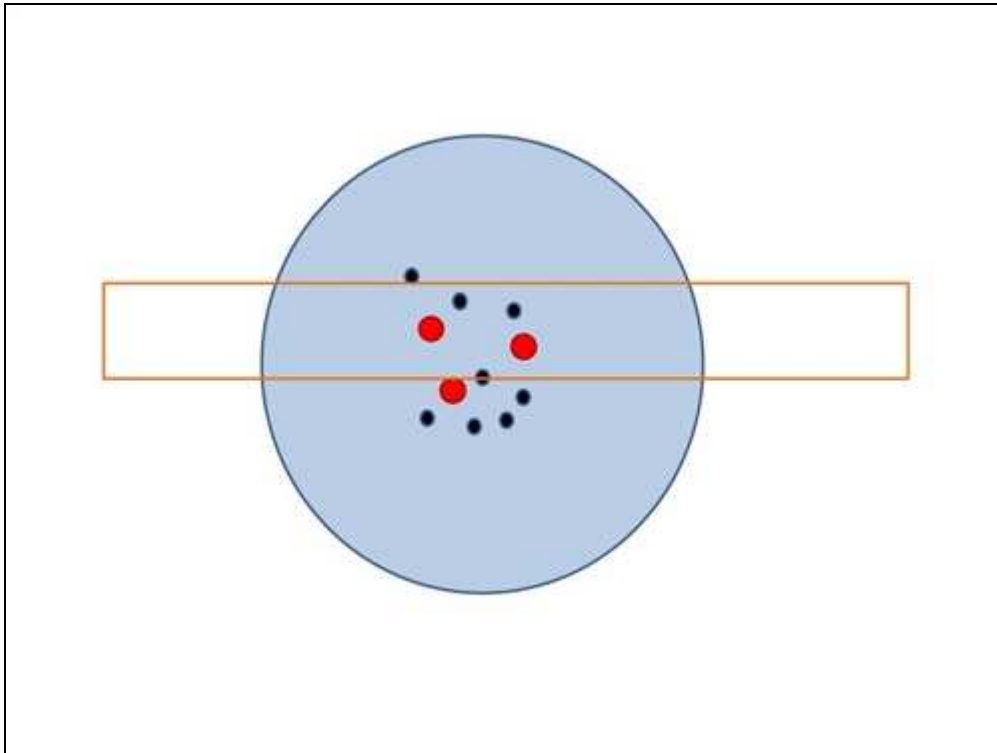






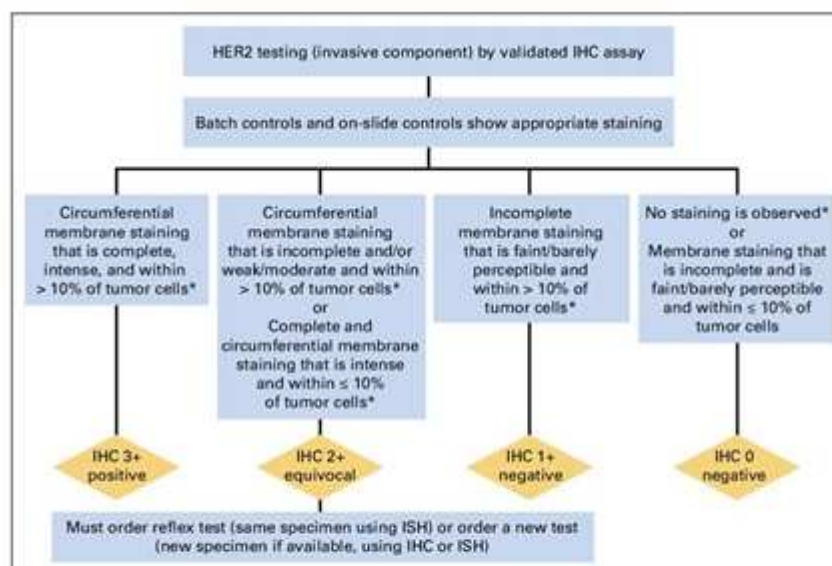
Lifelong Learning Programme

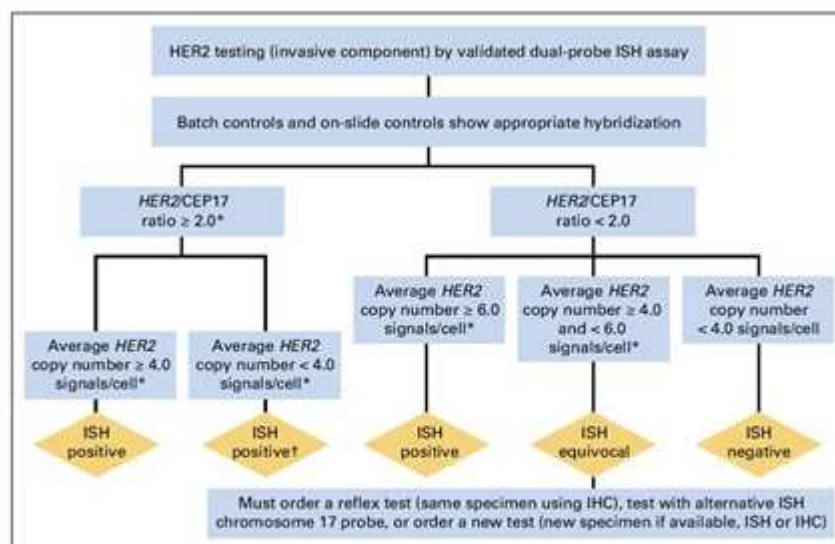




Problems

- Technical issues
- **Criteria**
- Interobserver/interlaboratory agreement
- heterogeneity





Svenska Bröstcancergruppens modifikation av ASCO CAP 2013 guidelines

- IHC 0, 1+, 2+, 3+
- ISH Kvot < 2, ≥ 2, ej utfört
- ISH HER2 copy nummer: <4, ≥4, ej utfört
- HER2 status: normal, amplifierat, ej utfört



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

HER2 IHC

- Intense
- Complete/circumferential
- > 10%

HER2 positive cases

- IH 3+
- ISH clusters
- ISH kvot ≥ 2
- Average HER2 copy number ≥ 4

ASCO-CAP criteria modified by the Swedish Breast Cancer Group



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Problems

- Gene-expression – protein expression discordance





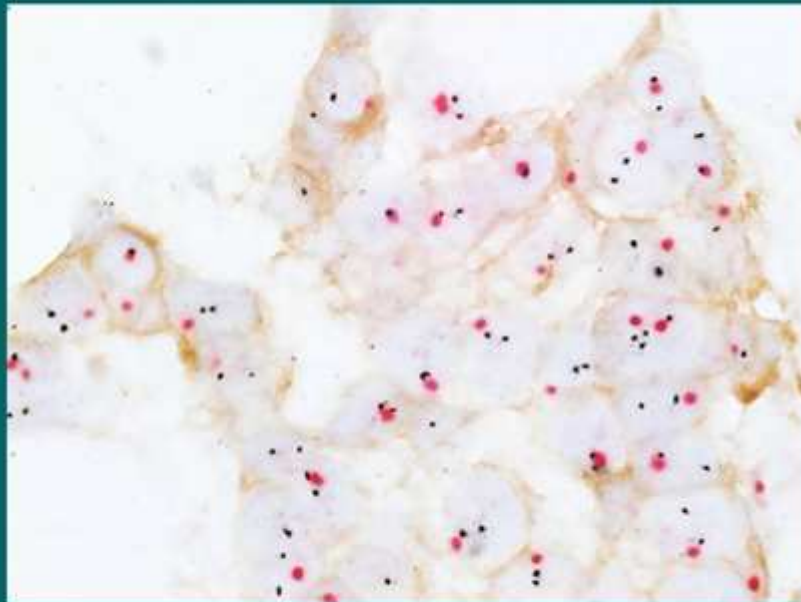
Lifelong Learning Programme



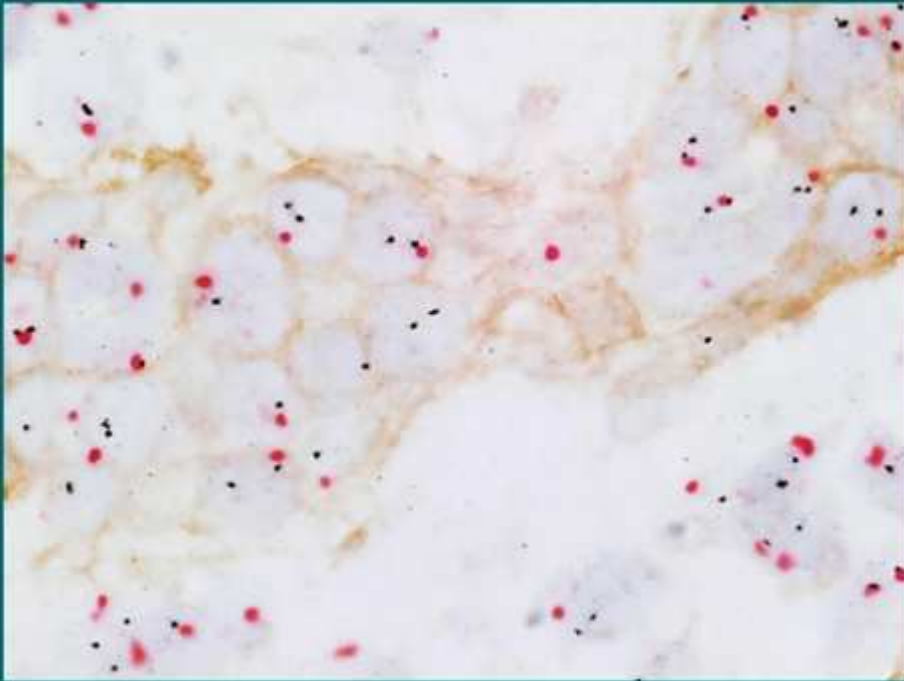


Problems

- monosomy



Monosomy of chr 17, non-amplified tumor

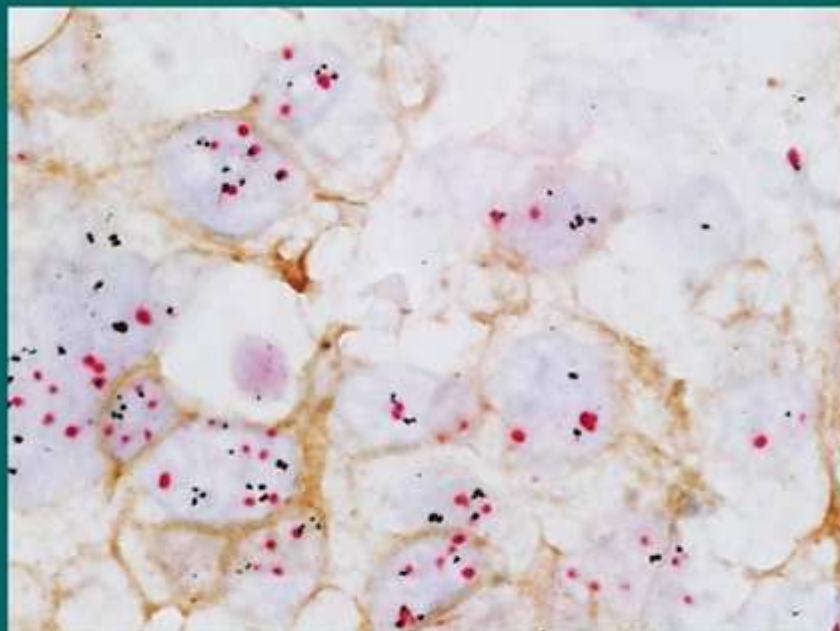


Monosomy of chr 17, non-amplified tumor



Problems

- polysomi



Polysomy of chr 17, non-amplified tumor



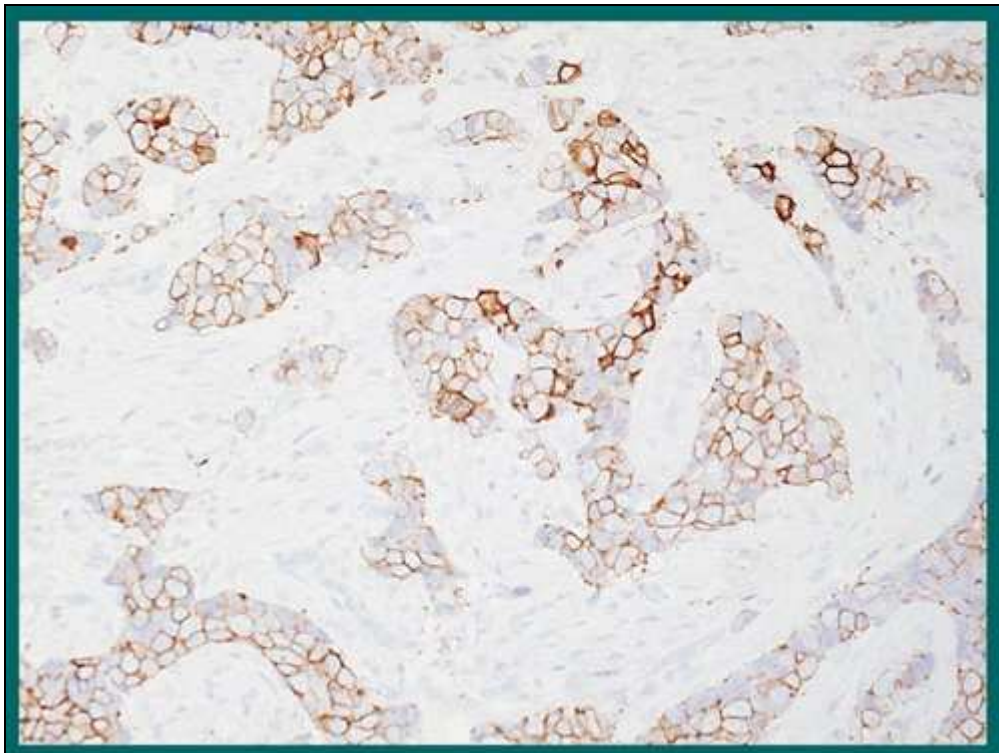
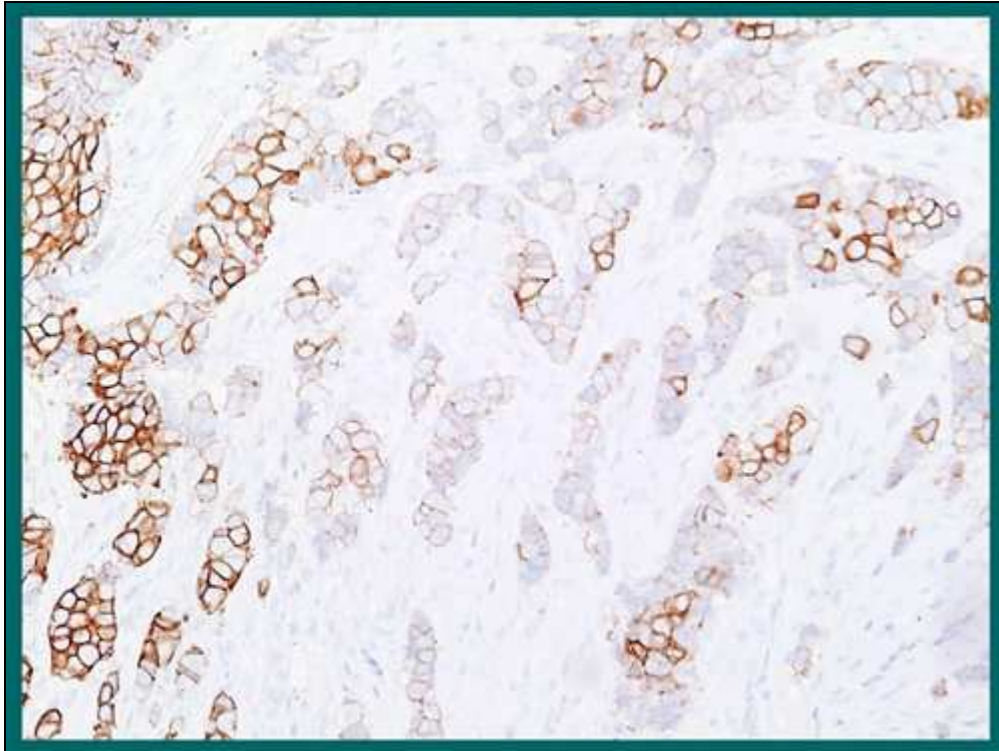
Polysomy of chr 17, non-amplified tumor

Problems

- heterogeneity



Lifelong Learning Programme



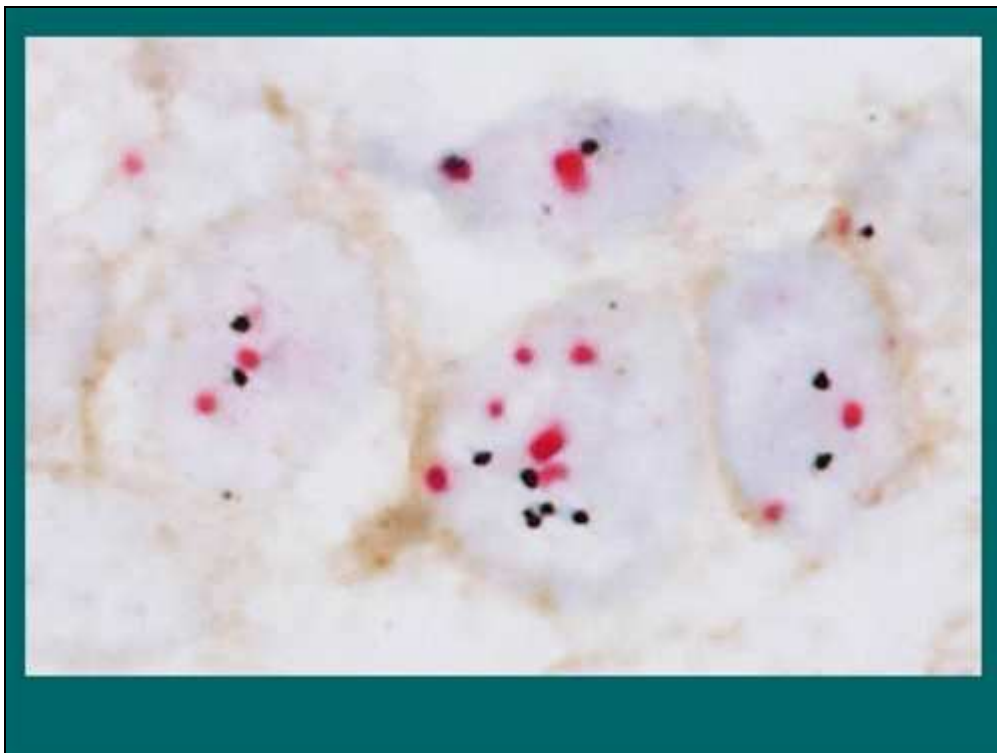
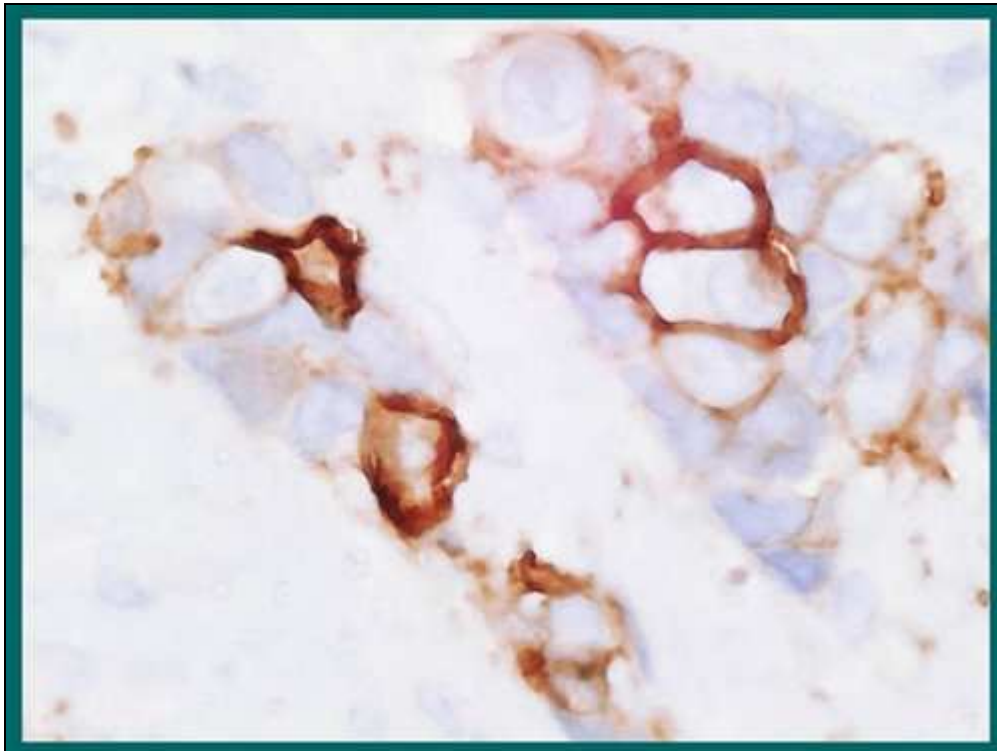


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Assessing heterogeneous cases

- IHC cut-off 10% (FDA recommendation)
- ISH >10 % of the cells are amplified

Wedad M Hanna, Josef Rüschoff, Michael Bilous, Renata A Coudry, Mitch Dowsett, Robert Y Osamura, Frédérique Penault-Llorca, Marc van de Vijver and Giuseppe Viale:
HER2 in situ hybridization in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. Modern Pathology 27, 4-18 (January 2014)

Assessing heterogeneous cases

- Prefer brightfield methods
- Scan the slide at low magnification
- Count 2-3 different fields
- At least 20 random cells/field

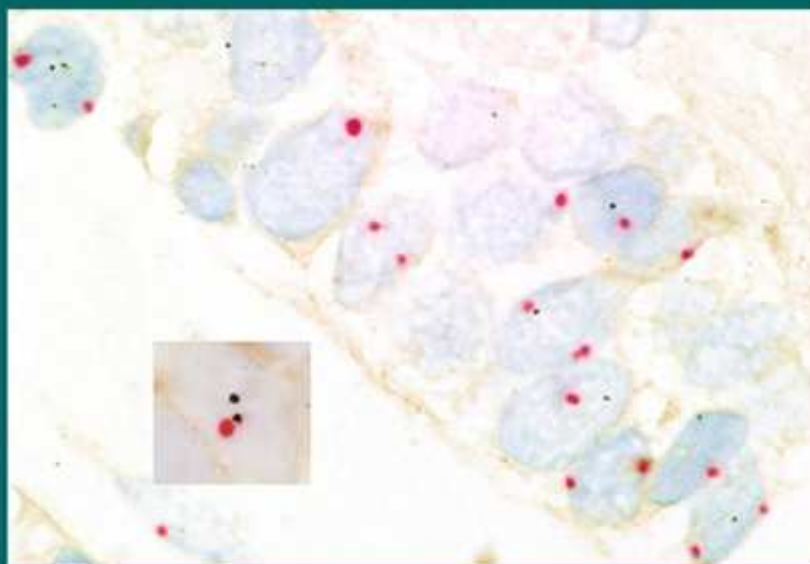
Wedad M Hanna, Josef Rüschoff, Michael Bilous, Renata A Coudry, Mitch Dowsett, Robert Y Osamura, Frédérique Penault-Llorca, Marc van de Vijver and Giuseppe Viale:
HER2 in situ hybridization in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. Modern Pathology 27, 4-18 (January 2014)



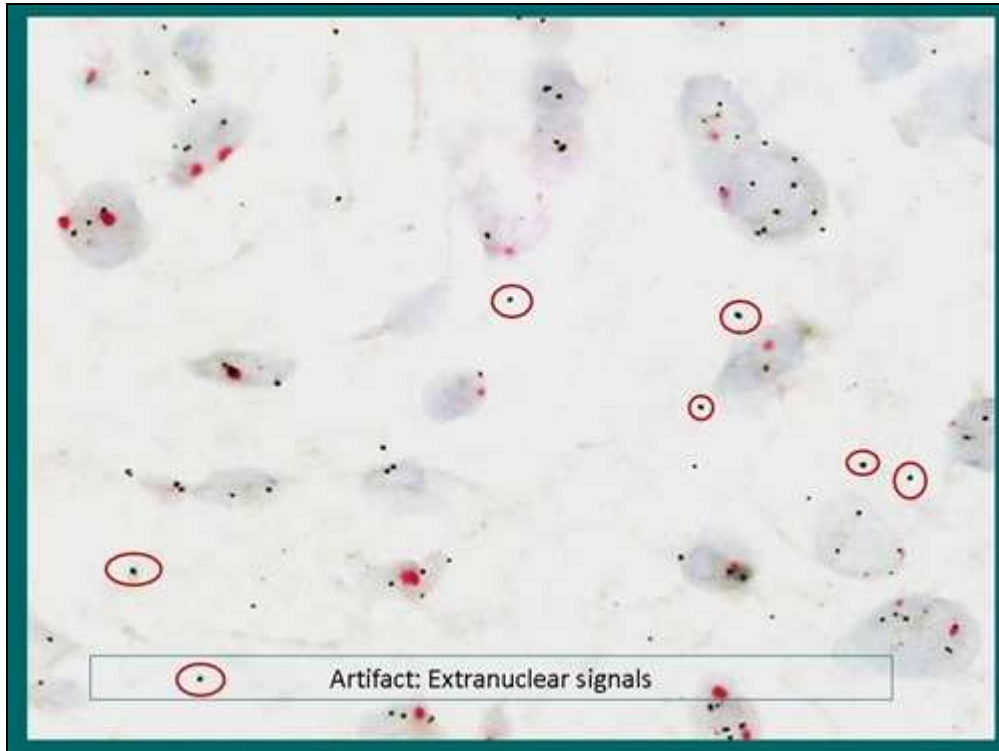
Lifelong Learning Programme

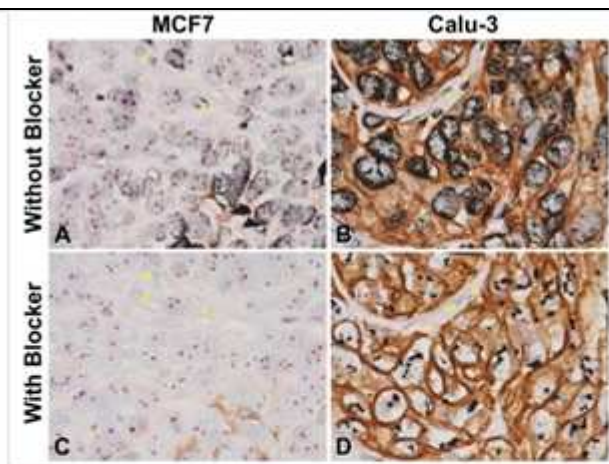


Artifacts



Artefact: very small HER-2 signals.





Naphthol phosphate blocks silver background staining resulting from the *HER2* & CEN17 BISH procedure. Images show *HER2* gene-protein staining results obtained without (A, B) or with naphthol phosphate (C, D) on formalin-fixed, paraffin-embedded (FFPE) *HER2*-negative MCF7 (A, C) and *HER2*-positive Calu-3 (B, D) xenograft tumors. In the absence of naphthol phosphate, non-specific silver deposition from the *HER2* BISH detection procedure obscures the BISH signals for the *HER2* gene and CEN17 targets (A, B) whereas the use of a BISH hybridization buffer containing naphthol phosphate eliminates the non-specific silver deposition (C, D). Some silver deposition was also seen in DAB staining (A). In the absence of naphthol phosphate, non-specific silver deposition occurred in mouse cells (yellow asterisks) (A, B) and mouse cells were confirmed without *HER2* and CEN17 BISH signals by using naphthol phosphate (C, D). 60 \times .

Diagn Pathol. 2012 May 30;7:60. doi: 10.1186/1746-1596-7-60.

A gene-protein assay for human epidermal growth factor receptor 2 (*HER2*): highfield tricolor visualization of *HER2* protein, the *HER2* gene, and chromosome 17 centromere (*CEN17*) in formalin-fixed, paraffin-embedded breast cancer tissue sections. Nitta IS, Kelly BD, Padilla M, Yock N, Brunschweiler E, Bai J, Smith S, Banner-Moore J, Baniari C, Tauda M, Grosan TM

	FISH (2008-2011)		Tricolor BDISH (2012-2013)	
	Amplified	Non-amplified	Amplified	Non-amplified
0, 1+	0/1	1/1	1/225	224/225
2+	13.7% (17/124)	86.3% (107/124)	10.4% (7/67)	89.6% (60/67)
3+	96.0% (48/50)	4.0% (2/50)	90.0% (36/40)	10.0% (4/40)
IH tested inv ca	96.7% (736/761)		98.8% (345/349)	
ISH tested	23.8% (175/736)		96.2% (332/345)	
% 2+	16.8% (124/736)		19.4% (67/345)	
% 3+	6.8% (50/736)		11.6% (40/345)	
HER2 +	9.1% (67/736)		13.9% (48/345)	

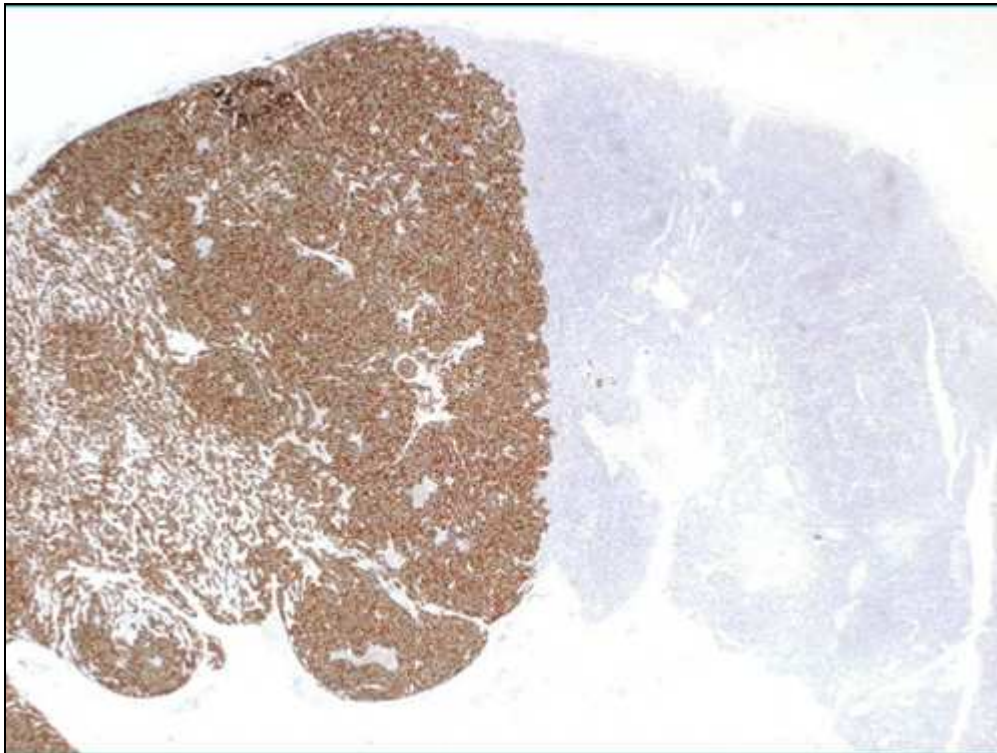


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Practical HER2 algorithm

- Standardized IH method
- Evaluation at low power magnification
- 3+ = HER-2 positive (10%)
- 0/1+ = HER-2 negative (70%)
- 2+ = ISH verification (20%)
- Heterogeneity = ISH



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Pitfalls created by artefacts in the diagnosis of breast cancer

**Isabella Castellano
Department of Medical Sciences
University of Turin, Italy
Breast Unit- Città della Salute e della Scienza
Hospital -Turin**

Prognostic markers in breast cancers

HISTOLOGICAL TYPE

HISTOLOGICAL GRADE

MARGIN STATUS

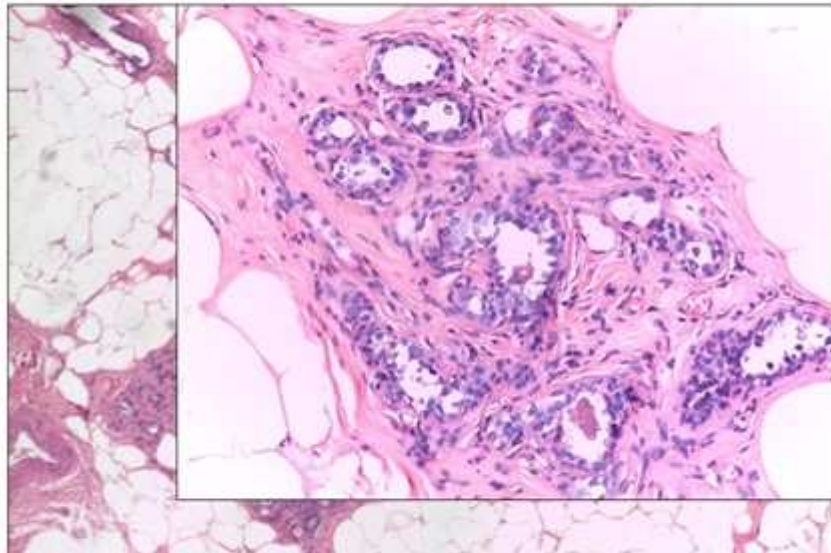


HISTOLOGICAL TYPE

CANCER OR NOT? ?

IN SITU OR INVASIVE?

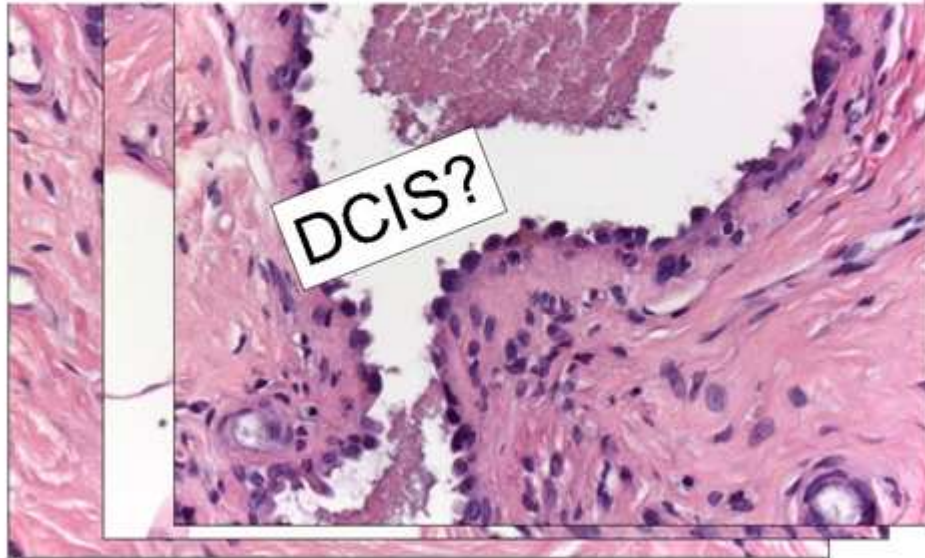
DUCTAL CARCINOMA OR SPECIAL TYPE?



Atypical epithelial cells in the terminal duct lobular unit, associated with variable degree of lobular sclerosis and atrophy



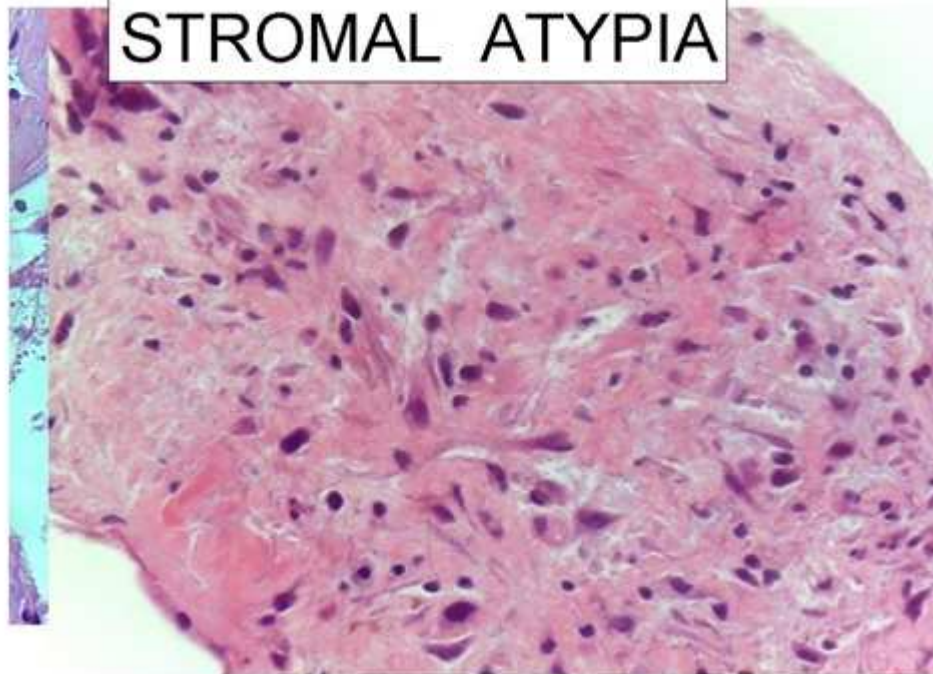
Large cells, with enlarged and diffusely hyperchromatic nuclei and small nucleoli. These cells often protrude into the lumen but do not show evidence of proliferation such as stratification, loss of polarity or mitosis. The distention of the ducts is not prominent.



EPITHELIAL ATYPIA

RADIATION THERAPY





HISTOLOGICAL TYPE

CANCER OR NOT?

IN SITU OR INVASIVE?

MICROINVASION/PSEUDOINVASION

DUCTAL CARCINOMA OR SPECIAL TYPE?



MICROINVASION-DEFINITION

Microinvasive carcinoma is defined as a tumour in which the dominant lesion is *in-situ* carcinoma (usually extensive high nuclear grade DCIS, rarely other types of DCIS or LCIS) but in which there are one or more, clearly separate, foci of infiltration usually into nonspecialized interlobular or interductal fibrous or adipose tissue, none measuring more than 1 mm (about 2 high power fields) in maximum diameter.

When there are multiple foci of MIC only the size of the largest focus is used to classify the microinvasion; the presence of multiple foci of microinvasion should however be noted and/or quantified.

Microinvasion is reported to be related to the size/extension of associated *in situ* carcinoma. The incidence rate of MIC ranges from 0.68% to 2.4%.

NB: A focus of invasive carcinoma 1 mm or less without associated *in situ* carcinoma is not a MIC but should be classified as invasive carcinoma and the maximum diameter measured

Diagnosis of microinvasion sometimes remains problematic, even with the use of ancillary techniques. If there is sufficient doubt about the presence of microinvasion (i.e. in cases with marked fibrosis or inflammation) the case should be classified as *in situ* carcinoma/microinvasion possible.

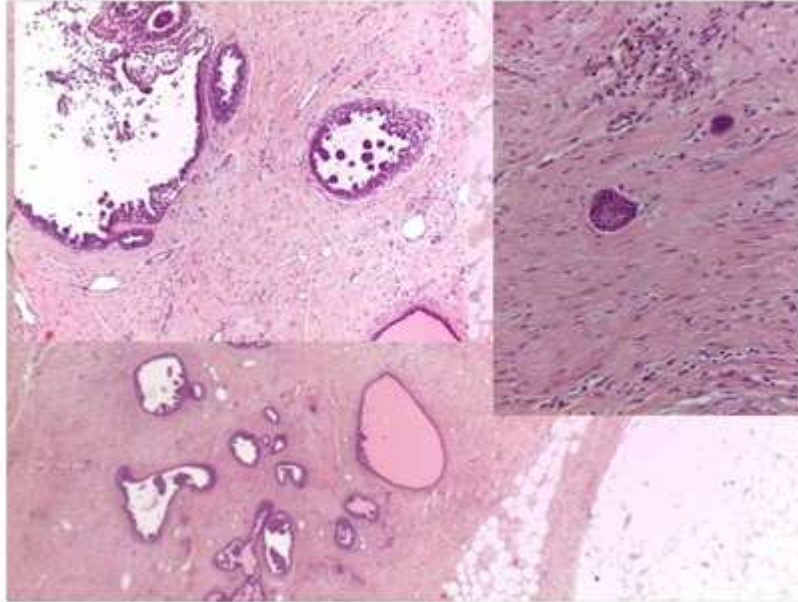
DIFFERENTIAL DIAGNOSIS

Microinvasion represents one of, if not the most, commonly overdiagnosed events in the pathology of breast carcinoma.

- 1) DCIS involving lobules ("lobular cancerization");
- 2) chronic inflammatory reaction present in association with, and obscuring, involved ducts and acini;
- 3) branching of ducts;
- 4) distortion or entrapment of involved ducts or acini by fibrosis (due to prior needling procedure);
- 5) crush artefacts;
- 6) Ca utery effects;
- 7) artefactual displacement of DCIS or LCIS cells into the surrounding stroma or adipose tissue due to tissue manipulation or a prior needling procedure
- 8) DCIS or LCIS involving benign complex sclerosing lesions such as radial scars, sclerosing adenosis, sclerosing papilloma, ductal adenoma.

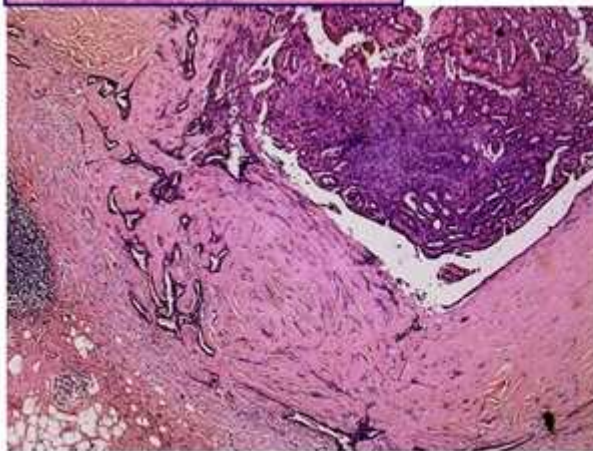
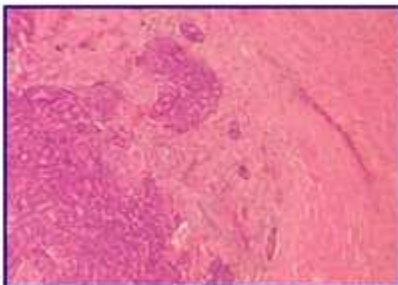


Artefactual displacement of DCIS cells into the surrounding stroma due to tissue manipulation or a prior needling procedure



PSEUDOINVASION

The tubules are within the sclerotic rim, they are confined to the scar of the needle biopsy site
Signs of hemorrhage are present
Cholesterol clefts may be seen





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

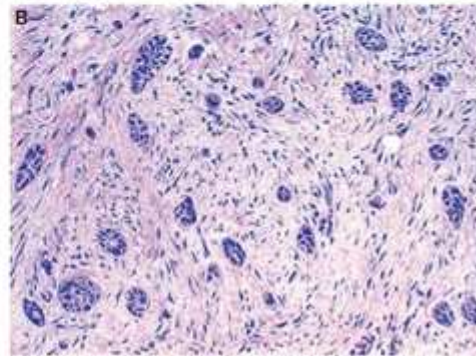
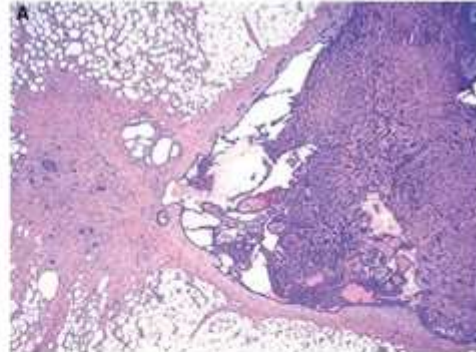
Histopathology 2008, 52, 20-29

displaced epithelium within
the core
needle biopsy site

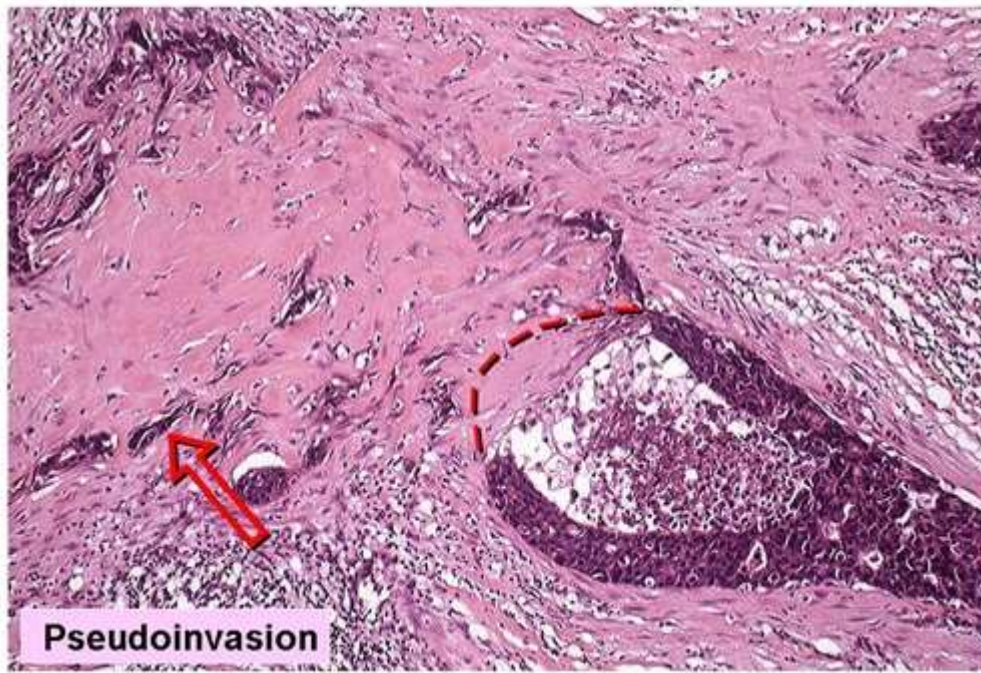
epithelial fragments or clusters are
confined to the organizing
haemorrhage, granulation tissue,
or scar of the needle biopsy site

epithelium that show varying
degrees of degenerative changes
and, not infrequently, squamoid
features may be seen in the
stroma

absence of myoepithelial cells must
not be used as evidence of an
invasive process

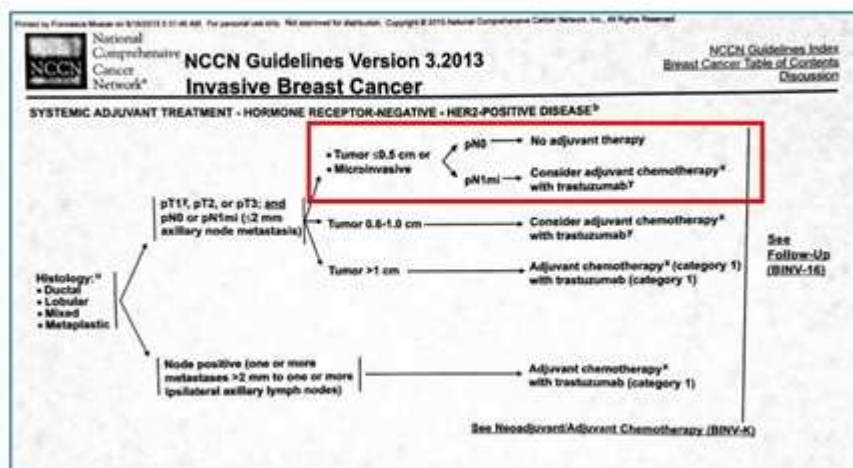


Tissue alteration following CB



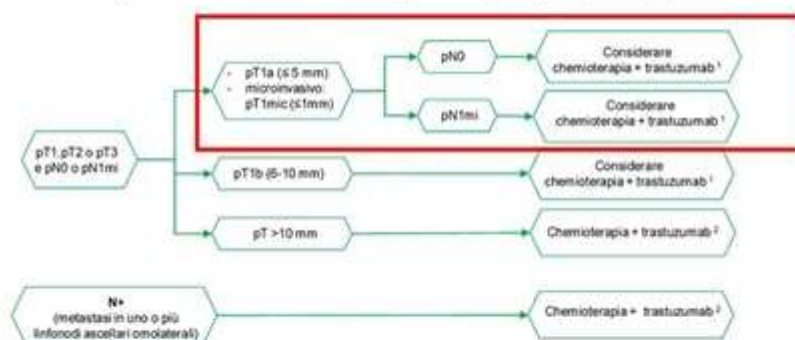


NCCN Guide Lines



ALGORITMO 7 – CARCINOMA MAMMARIO INFILTRANTE

Terapia sistemica adiuvante: ER negativi e PgR negativi; HER2-positivo



Nota 1 - Nei tumori microinvasivi e nei tumori pT1a e pT1b, pN0/pN1mi non esistono dati prospettici relativi al beneficio del trastuzumab adiuvante. Si può prendere in considerazione la chemioterapia e il trastuzumab tenendo presenti nella scelta anche il G, il Ki-67, l'età e le comorbidità della paziente (vedere paragrafo 4.2.2.3).

Nota 2 - Per i tumori di diametro superiore ad un centimetro o per i tumori N+, è indicato trattamento sistemico adiuvante con chemioterapia e trastuzumab.



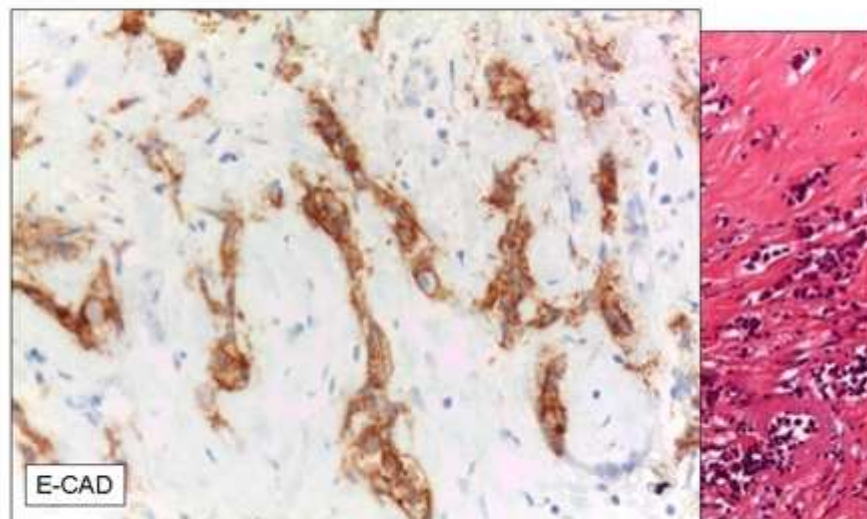
HISTOLOGICAL TYPE

CANCER OR NOT?

IN SITU OR INVASIVE?

DUCTAL CARCINOMA OR SPECIAL TYPE?

LOBULAR OR DUCTAL?



Poor fixation can induce stromal architectural and cytological atypia



LOBULAR CARCINOMA



PRE-OPERATORY: MAGNETIC RESONANCE

EUROPEAN JOURNAL OF CANCER 46 (2 0 1 0) 1 2 9 6 –1 3 1 6

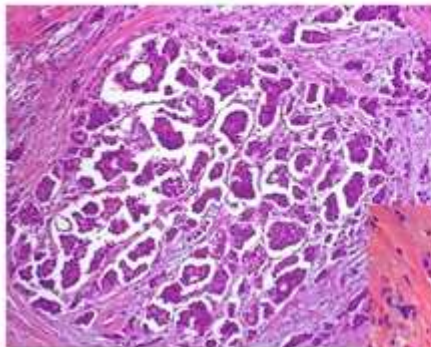
Magnetic resonance imaging of the breast: Recommendations from the EUSOMA working group
Sardanelli et al.

POST-OPERATORY:

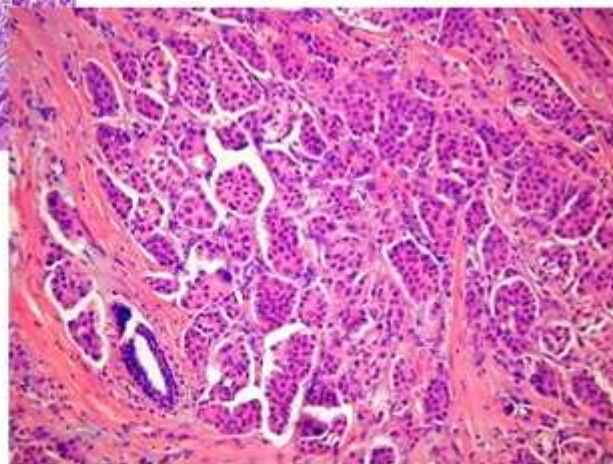
- LOCAL AGGRESSIVENESS
- LESS RESPONSIVE TO CHEMOTHERAPY

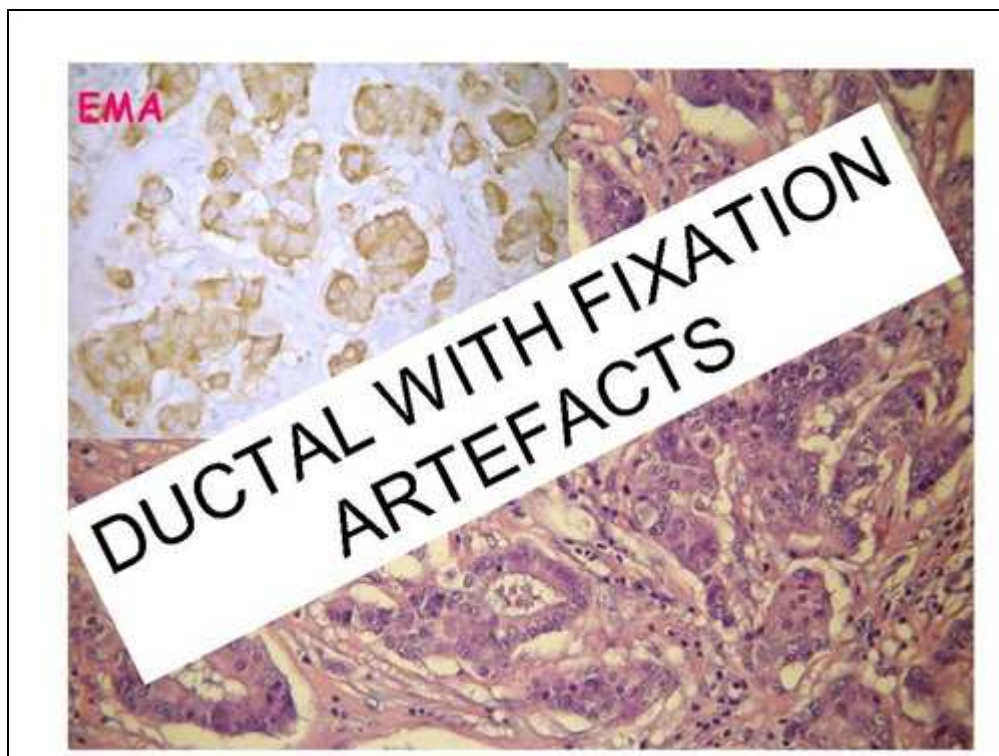
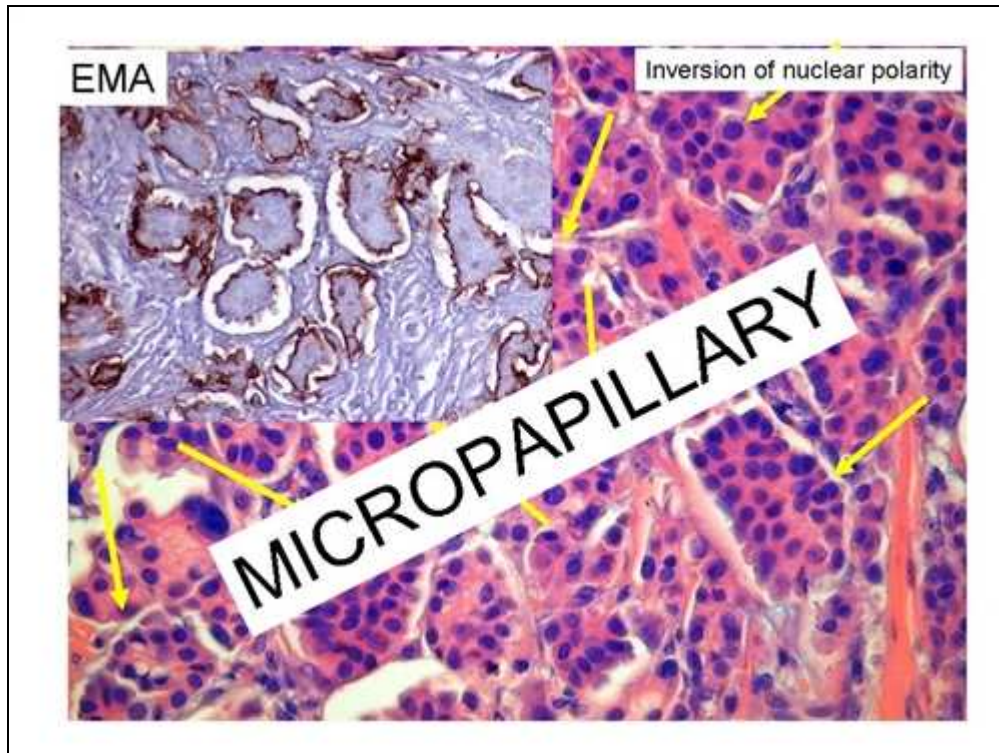
Breast cancer Res Treat 2014 Feb;144(1):153-62
Eur J Surg Oncol, 2003 29(4): 361–367
Ann Oncol, 2006 17(8): 1228–1233
Breast J, 2009 15(2): 146–154
British Journal of Cancer (2013) 108, 285–291

MICROPAPILLARY OR DUCTAL?



Neoplastic glands surrounded by empty space







MICROPAPILLARY CARCINOMA

MPCs were characterized by a high prevalence of extensive peritumoral vascular invasion, MPC patients were also characterized by a prevalence of regional metastases significantly higher than those with ductal carcinoma NOS, with a large proportion of cases involving four or more lymph nodes.



Table 4. Available data on micropapillary carcinoma patients survival

	Pts #	Recurrence		Dead of disease		Clinical relevance	Matched with ICC-NOS
Tavazzoli <i>et al.</i> ¹⁵	5	1 (20%)	At 32 months	4 (80%)	41-56 months	No	No
Maddipati <i>et al.</i> ¹⁶	10	9 (90%)	Mean: 24 months	5 (50%)	Mean: 55 months	Yes	No
Trevisan <i>et al.</i> ¹⁴	15	4 (27%)	Mean: 34.3 months	4 (27%)	Mean: 43 months	Yes	No
Petrakis <i>et al.</i> ¹⁷	21	—	—	—	—	No	Yes
Luna-More <i>et al.</i> ¹⁸	54	—	—	20 (37%)	Mean: 59 months	Yes	No
Nasser <i>et al.</i> ¹⁹	83	5 (16%)	Mean: 117 months	38 (46%)	Mean: 84 months	No	Yes
Karada <i>et al.</i> ¹⁷	17	—	—	10 (59%)	Mean: 72 months	Yes	No
Petlinidis <i>et al.</i> ¹⁸	62	29 (71%)	Mean: 30 months	20 (49%)	1-10.5 years	Yes	No
Yu <i>et al.</i> ⁶	72	15 (20.8%)	Mean: 26 months	10 (14%)	Mean: 45 months	NR	Yes

Histopathology 2013, 63, 217–224. DOI: 10.1111/his.12147

Prognostic markers in breast cancers

HISTOLOGICAL TYPE

HISTOLOGICAL GRADE

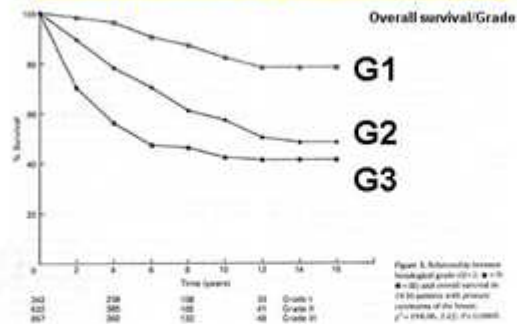


MARGIN STATUS



HISTOLOGICAL GRADE

Histopathology 1991;19, 403

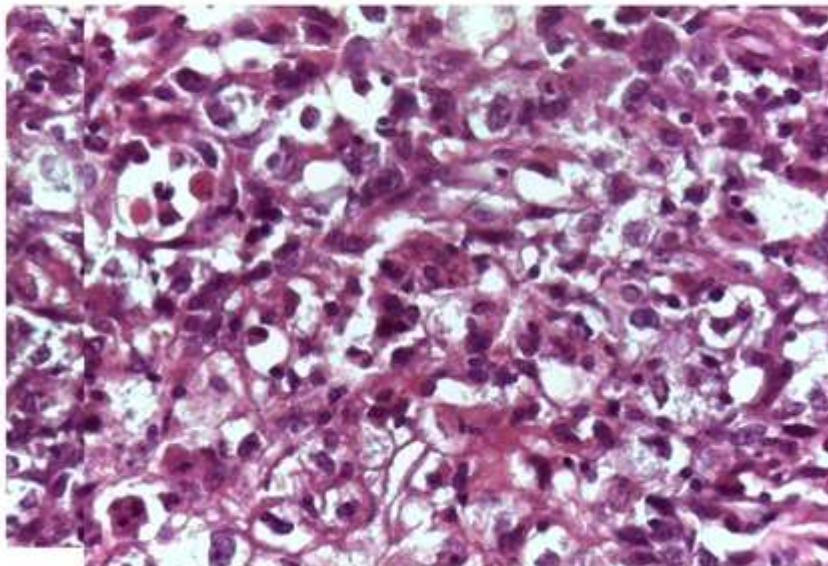


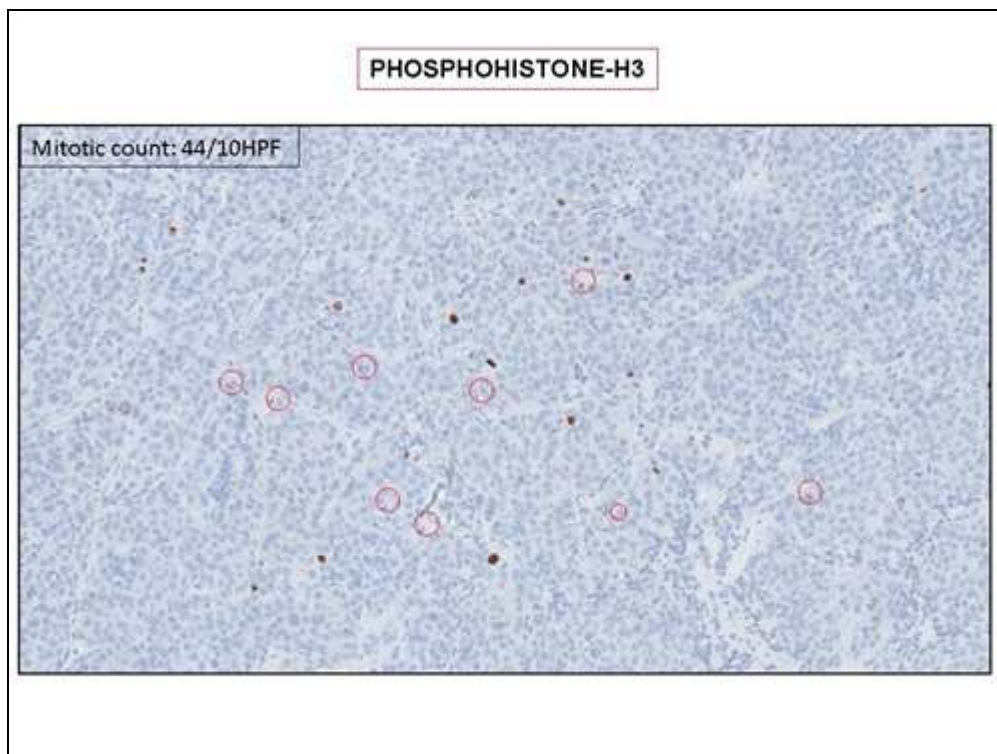
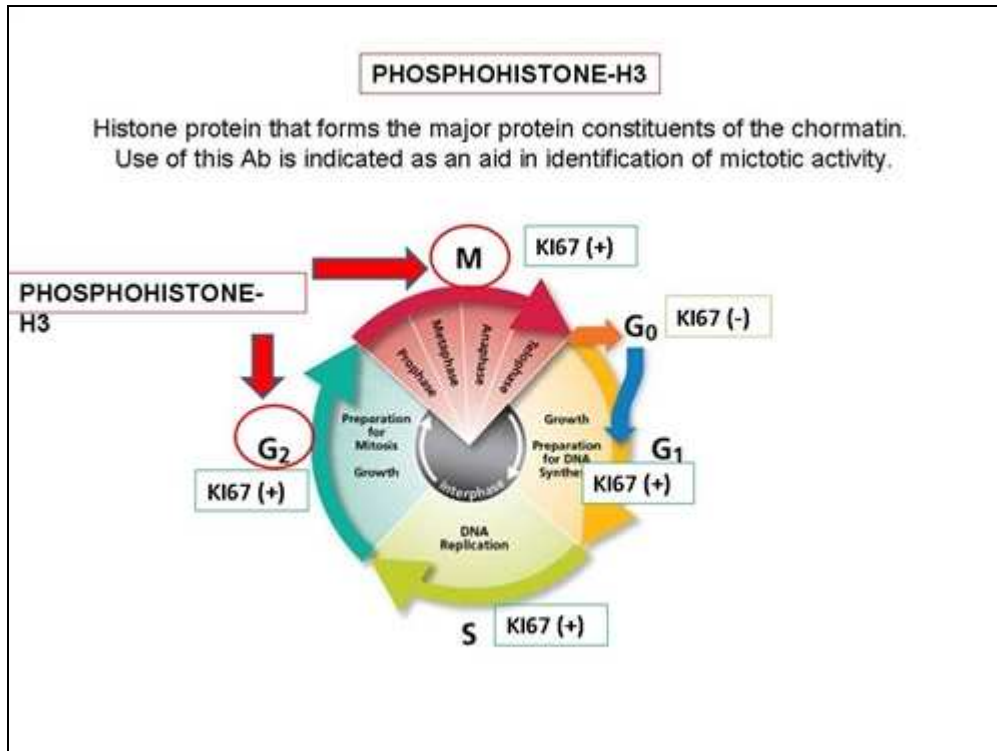
MITOTIC COUNT

TUBULAR FORMATION

NUCLEAR PLEOMORPHISM

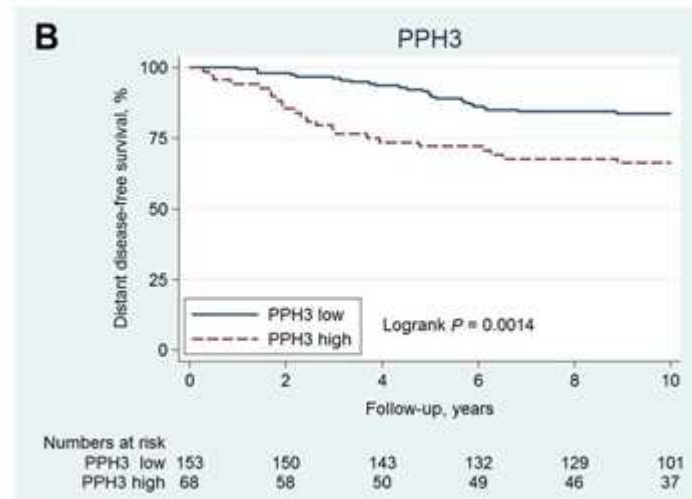
MITOTIC COUNT/ APOPTOSIS





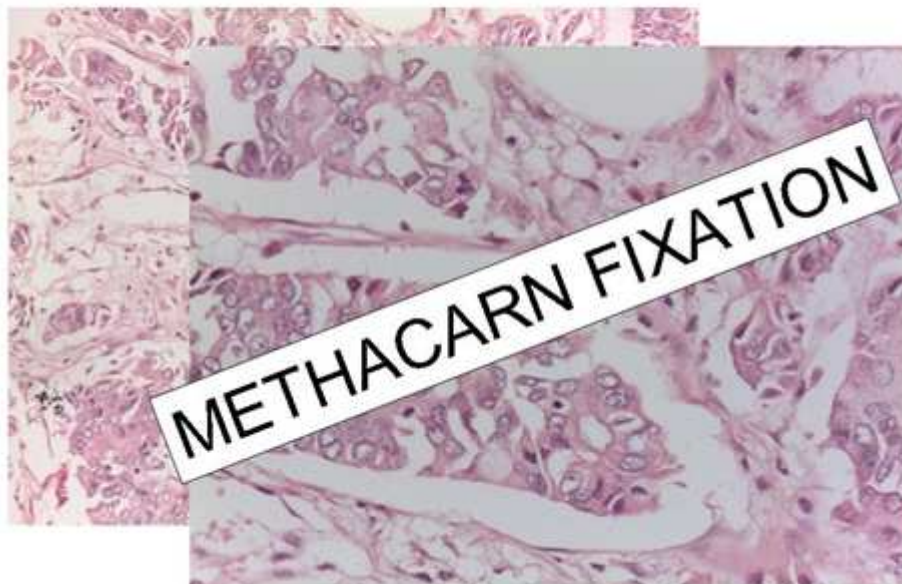


PPH3 is a strong prognostic marker in node-negative breast cancer, specifically in ER-positive patients and patients with histological grade 2.



PLOS ONE | December 2013 | Volume 8 | Issue 12 | e81902

NUCLEAR PLEOMORPHISM:





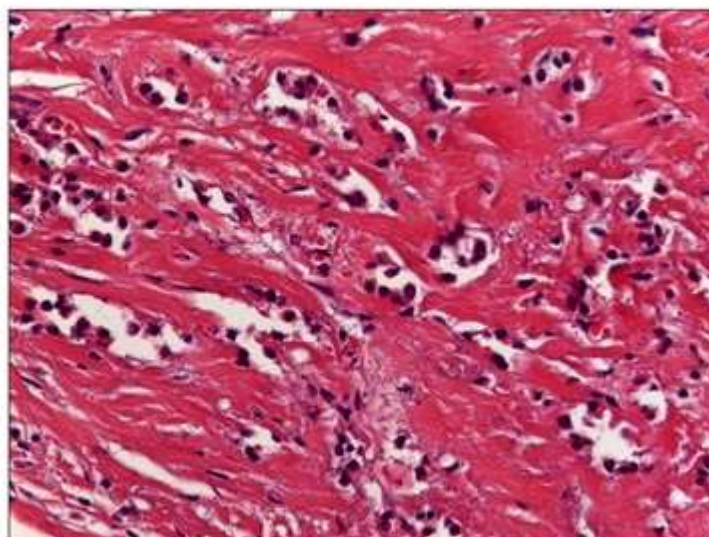
Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

TUBULE FORMATION



American Society of Clinical Oncology/College of American
Pathologists Guideline Recommendations for
Immunohistochemical Testing of Estrogen and
Progesterone Receptors in Breast Cancer JCO 2010

Preanalytic standardization:

- *Sample slicing* at 5-mm intervals after appropriate gross inspection and margins designation
- *type of fixative*. **Only 10% NBF** should be used as the fixative for breast tissue specimens. Higher or lower concentrations of NBF are not acceptable
- *duration of tissue fixation*. Breast tissue specimens must be fixed in 10% NBF for **no less than 6 hours** and for **not more than 72 hours** before processing



American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer JCO 2010

Preanalytic standardization:

- *time from tissue acquisition* (defined as the time that the tissue is handed from the surgical field) *to fixation* should be as short as possible and must be recorded. The time from tumor removal to fixation should be kept to 1 hour to comply with these recommendations.
- The pathologist should effectively communicate this priority to all members of the breast care management team so processes are put in place to make sure these times are routinely recorded.
- It is the responsibility of the surgeon and operating room staff or the radiologist and his/her staff obtaining the specimen to document the collection time, and
- it is the responsibility of the pathologist and laboratory staff to document the fixation start time.
- if tumor comes from *remote location*, it should be bisected through the tumor on removal and sent to the laboratory immersed in a sufficient volume of NBF

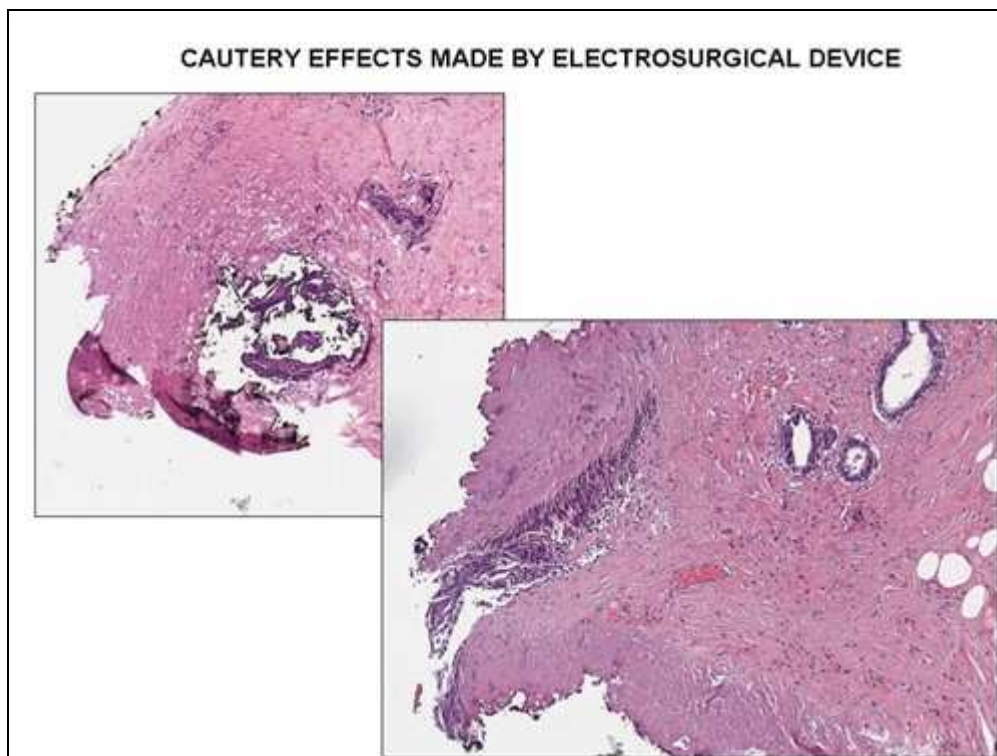
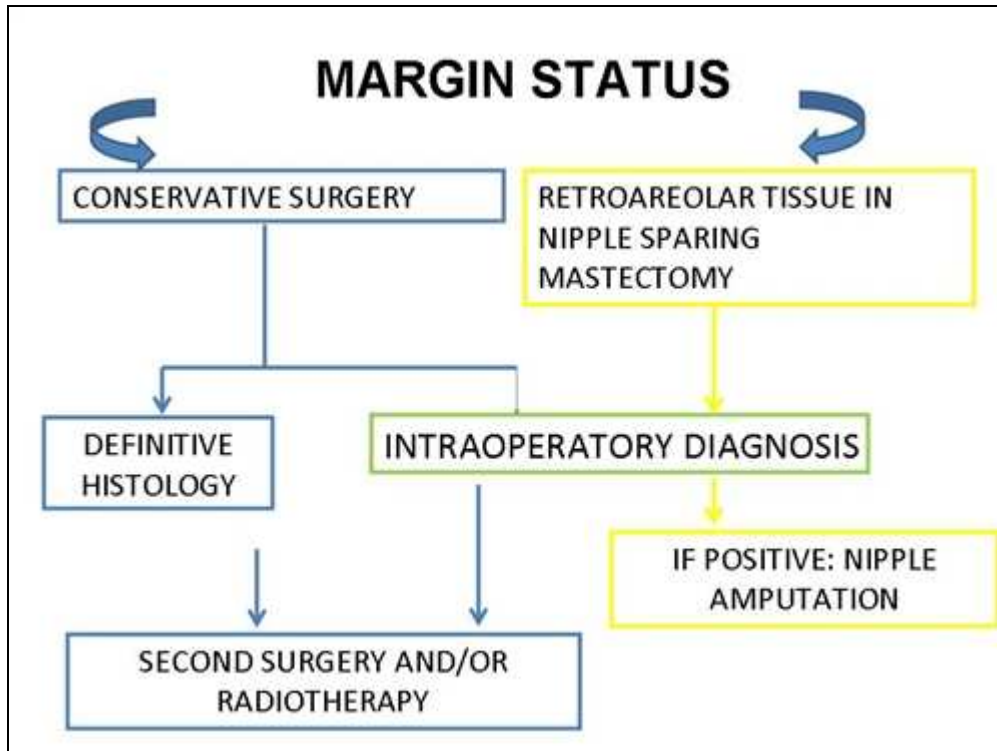
Prognostic markers in breast cancers

HISTOLOGICAL TYPE

HISTOLOGICAL GRADE

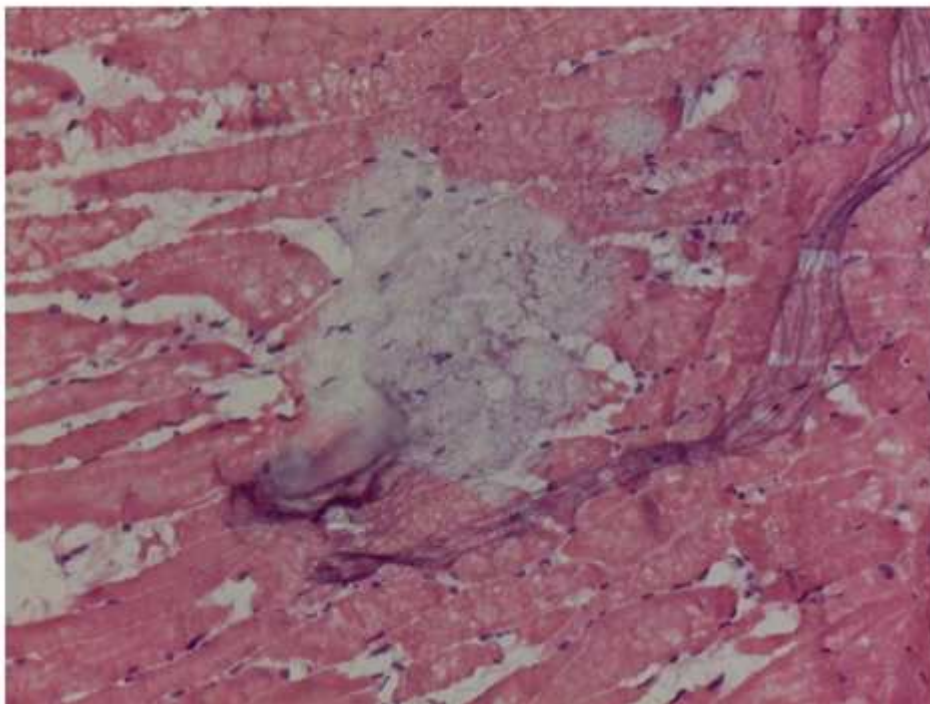
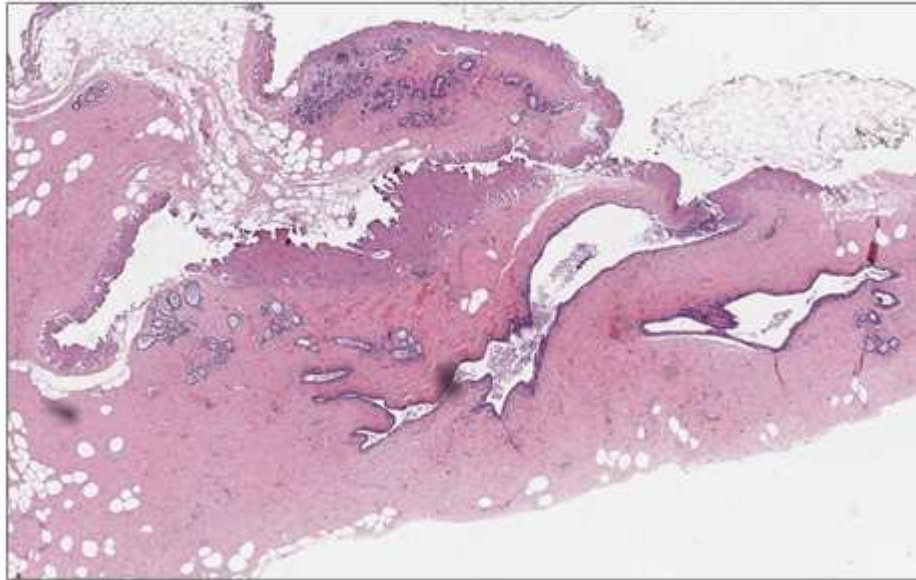
MARGIN STATUS







Lifelong Learning Programme





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

CONCLUSIONS

To avoid pitfalls created by artefacts

CLINICAL HISTORY

FORMALIN FIXATION

MULTIDISCIPLINARY APPROACH



THANK YOU FOR YOUR ATTENTION



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



The Fourth TASTE Workshop

Pitfalls and Algorithms in Diagnostic Pathology

Pitfalls in immunohistochemical procedures

Gianni Bussolati

COREP & Univ. of Torino

Sources of pitfalls in IHC

- **Fixation**
 - Tissue processing and embedding
 - Decalcification
 - Antigen retrieval
 - Primary antibodies
 - Endogenous enzyme activity
 - Effects of Avidin / Biotin
 - Detection system
 - Chromogen
 - Results interpretation

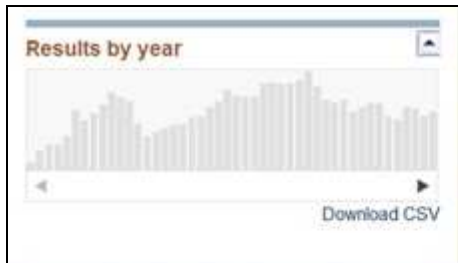


Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



**PubMed Query: Fixation and Immunohistochemistry:
Results: 9470**

J Clin Pathol. 2014 Apr 15.

The effect of delay in fixation on HER2 expression in invasive carcinoma of the breast assessed with immunohistochemistry and in situ hybridisation.

Lee AH¹, Key HP, Beil JA, Kumar P, Hodi Z, Ellis IO.

Am J Clin Pathol. 2003;120:86-92.

**Minimum formalin fixation time for
consistent estrogen receptor
immunohistochemical staining of invasive
breast carcinoma.**

**Goldstein NS, Ferkowicz M,
Odish E, Mani A, Hastah F.**

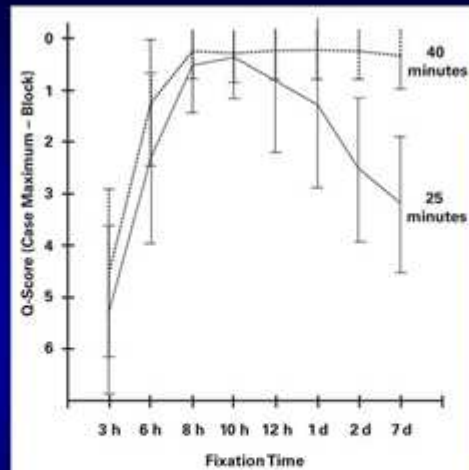


Figure 1 Plateau, case maximum (max) estrogen receptor (ER) scores occurred at 6-8 hours. Standard immunohistochemical method used 40 minutes of antigen retrieval.

Goldstein NS et al. Am J Clin Pathol. 2003;120:86-92.

Fixation in Formalin (for IHC)

- Better overfixation than underfixation
- (Related to the size of tissue block)



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Recommendations for IHC

-Fixation:

Formalin (alternatives: not reliable).

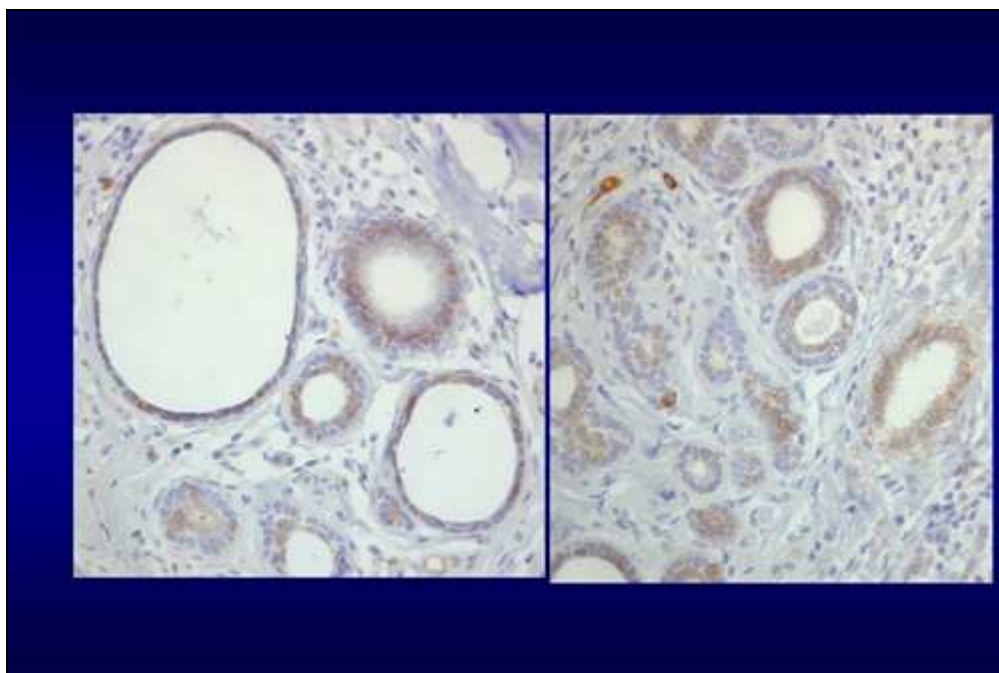
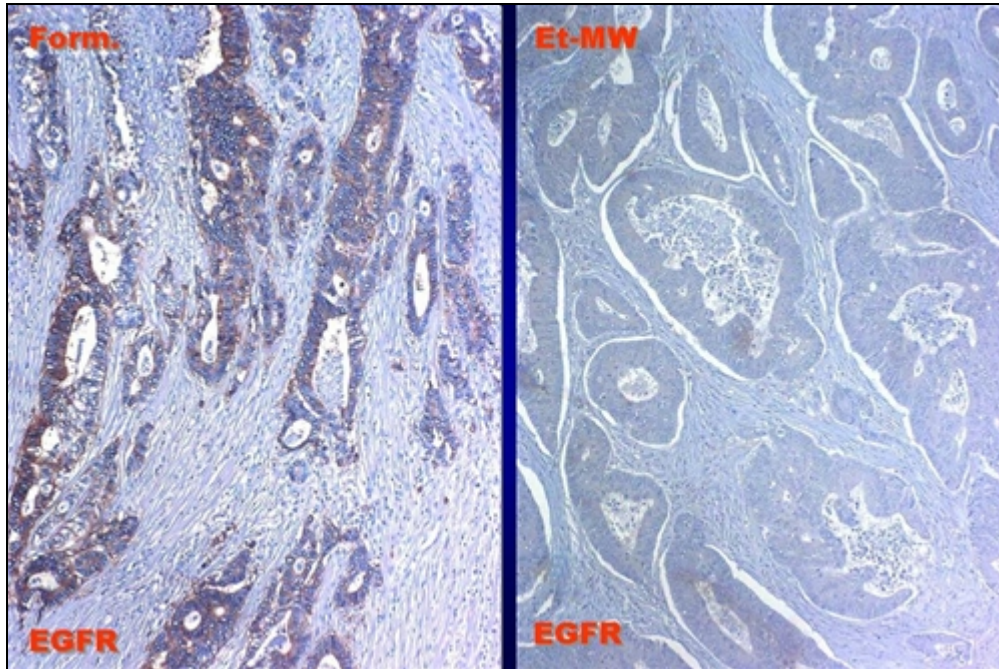
- Time: 8-12 h.
- Diffusion rate: 0,79
(Approx. 1 mm/h).
- Difference between penetration and fixation

Factors Affecting IHC Staining

a) Fixation

Cause	Effect	Reference
Under-fixation with formalin	Reduced immuno-staining in central areas of sections	1, 11, 12
Over-fixation with formalin	Non-specific binding of antibodies by free-aldehyde group	3
Alcohol fixation	Most of CD and some growth factors peptides are poorly reactive	13-15
Mercury-based fixatives (B5, Zenkers)	CD4, CD5, CD10, CD23, (CD30) loose immuno-reactivity	14-16

Bussolati G and Leonardo E. J Clin Pathol. 2008,





Sources of pitfalls in IHC

- Fixation
- **Tissue processing and embedding**
- Decalcification
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- Effects of Avidin / Biotin
- Detection system
- Chromogen
- Results interpretation

Factors Affecting IHC Staining

a2) tissue processing

Cause	Effect	Reference
Dehydration, non-polar solvents and paraffin embedding	Possible change on the conformation of some antigens	18-20
Decalcification by 10% formic acid or by 5% nitric acid	Decreased antigenicity on many antigens, particularly CD markers	21-23, 26-28

Bussolati G and Leonardo E. J Clin Pathol. 2008,



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

J Histochem Cytochem. 1985;33:1103-9.

**Influence of fixation and decalcification on the
immunohistochemical staining of
cell-specific markers in paraffin-embedded
human bone biopsies**

Mullink H, Henzen-Logmans SC,
Tadema TM, Mol JJ, Meijer CJ.

**“....best demonstrated formalin-fixed
and acetic acid-decalcified biopsies.”**

Bone marrow

MolDecal leads to excellent preservation of DNA and allows
FISH analysis in the majority of cases !!

Problems in immunohistochemistry involve primarily CD20,
CD117 and CD138 detection

Anagnostopoulos J, 2014



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- **Antigen retrieval**
 - Primary antibodies
 - Endogenous enzyme activity
 - Effects of Avidin / Biotin
 - Detection system
 - Chromogen
 - Results interpretation

Antigen Retrieval (A.R.)

-Specify:

-Time

-Buffer

-Heating procedure

-Enzymes

-Treatment to be related to fixation time (and to time of paraffin embedding).

Factors Affecting IHC Staining

Cause	Effect	Reference
PIER at incorrect pH and/or temperature	Antigens not retrieved (masked)	39-44
PIER with strong enzymatic digestion	Potential destruction of antigens	42-44
HIER at pH3.0-6.0	Decrease in staining of Ki67 (Mib1) and ER	49
HIER on biotin-rich tissues (e.g. mitochondrion-rich cells)	Unmasking of endogenous biotin	59, 80-84
HIER by zinc sulphate, citrate (pH6) and TRIS (pH9) buffer solutions	Non-specific staining of nuclear proteins	88-90

Bussolati G and Leonardo E. J Clin Pathol. 2008.

[illegible]

Year 1990	1990	1990	1990	1990	1990
Year 1991	1991	1991	1991	1991	1991
Year 1992	1992	1992	1992	1992	1992
Year 1993	1993	1993	1993	1993	1993
Year 1994	1994	1994	1994	1994	1994
Year 1995	1995	1995	1995	1995	1995
Year 1996	1996	1996	1996	1996	1996
Year 1997	1997	1997	1997	1997	1997
Year 1998	1998	1998	1998	1998	1998
Year 1999	1999	1999	1999	1999	1999
Year 2000	2000	2000	2000	2000	2000
Year 2001	2001	2001	2001	2001	2001
Year 2002	2002	2002	2002	2002	2002
Year 2003	2003	2003	2003	2003	2003
Year 2004	2004	2004	2004	2004	2004
Year 2005	2005	2005	2005	2005	2005
Year 2006	2006	2006	2006	2006	2006
Year 2007	2007	2007	2007	2007	2007
Year 2008	2008	2008	2008	2008	2008
Year 2009	2009	2009	2009	2009	2009
Year 2010	2010	2010	2010	2010	2010
Year 2011	2011	2011	2011	2011	2011
Year 2012	2012	2012	2012	2012	2012
Year 2013	2013	2013	2013	2013	2013
Year 2014	2014	2014	2014	2014	2014
Year 2015	2015	2015	2015	2015	2015
Year 2016	2016	2016	2016	2016	2016
Year 2017	2017	2017	2017	2017	2017
Year 2018	2018	2018	2018	2018	2018
Year 2019	2019	2019	2019	2019	2019
Year 2020	2020	2020	2020	2020	2020
Year 2021	2021	2021	2021	2021	2021
Year 2022	2022	2022	2022	2022	2022
Year 2023	2023	2023	2023	2023	2023
Year 2024	2024	2024	2024	2024	2024
Year 2025	2025	2025	2025	2025	2025
Year 2026	2026	2026	2026	2026	2026
Year 2027	2027	2027	2027	2027	2027
Year 2028	2028	2028	2028	2028	2028
Year 2029	2029	2029	2029	2029	2029
Year 2030	2030	2030	2030	2030	2030
Year 2031	2031	2031	2031	2031	2031
Year 2032	2032	2032	2032	2032	2032
Year 2033	2033	2033	2033	2033	2033
Year 2034	2034	2034	2034	2034	2034
Year 2035	2035	2035	2035	2035	2035
Year 2036	2036	2036	2036	2036	2036
Year 2037	2037	2037	2037	2037	2037
Year 2038	2038	2038	2038	2038	2038
Year 2039	2039	2039	2039	2039	2039
Year 2040	2040	2040	2040	2040	2040
Year 2041	2041	2041	2041	2041	2041
Year 2042	2042	2042	2042	2042	2042
Year 2043	2043	2043	2043	2043	2043
Year 2044	2044	2044	2044	2044	2044
Year 2045	2045	2045	2045	2045	2045
Year 2046	2046	2046	2046	2046	2046
Year 2047	2047	2047	2047	2047	2047
Year 2048	2048	2048	2048	2048	2048
Year 2049	2049	2049	2049	2049	2049
Year 2050	2050	2050	2050	2050	2050

Bussolati G, Leonardo EJ Clin Pathol. 2008;

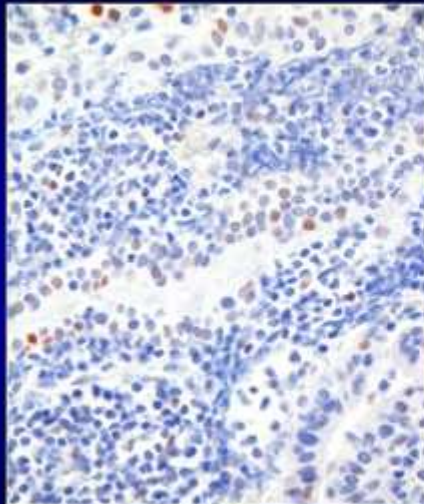


Education and Culture DG

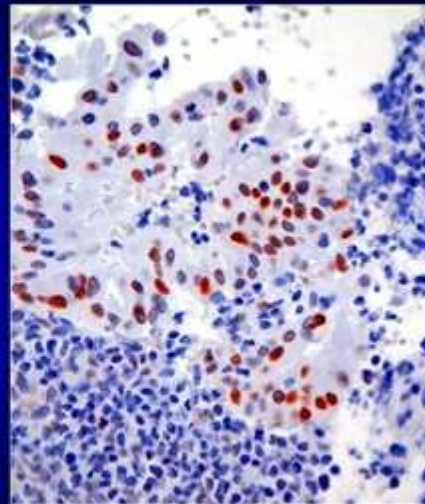
Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



ER A.R. at pH3



ER A.R. at pH6

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- **Primary antibodies**
 - Endogenous enzyme activity
 - Effects of Avidin / Biotin
 - Detection system
 - Chromogen
 - Results interpretation



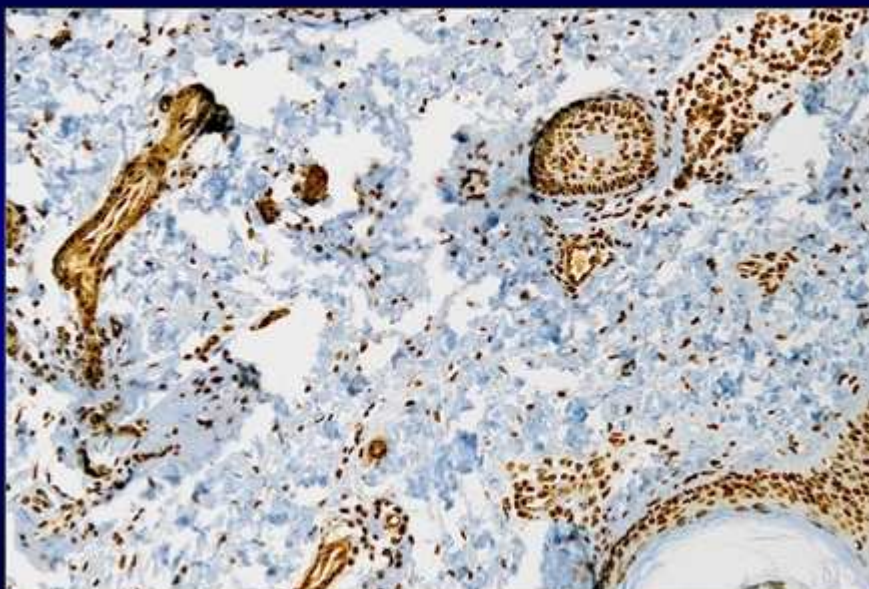
Factors Affecting IHC Staining

e) Primary antibody

Cause	Effect	Reference
Improper Reaction buffer	Background staining or absence of antigen binding	19, 29, 99, 100
Hydrophobicity, polymerization and aggregation of the immunoglobulins	High Background staining	104, 105
Interaction with protein polar groups in tissue sections	High Background staining	104
Protein-antibody complement-mediated binding	High Background staining	106
Attraction of the Fc fragment to basic groups of collagen fibers	Non-specific staining	107
Binding of immunoglobulins to the cellular Fc receptors (19, 108).	Non-specific staining	19, 106
High concentrations of antibodies	Background staining or absence of antigen binding (prozone phenomenon)	109
Bacterial contamination of antibodies	Antibody agglutination	100

Bussolati G and Leonardo E. J Clin Pathol. 2008,

Actin + nuclei 1.jpg





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Goldstein et al. Appl Immunolistochem Mol Morphol. 2007, 15:124-33

TABLE 2. List of Factors That Could be Adjusted During the Antibody Optimization

Parameter	Description
No pretreatment	Some antibodies still perform best without any type of pretreatment
Enzyme digestion	Few antibodies perform best only when enzyme digestion was used without the need for heat induced epitope retrieval
Retrieval buffer	The combination of the type of buffer (ie, citration, ethylenediaminetetraacetic acid, trishydroxymethylaminomethane), and pH level can result in dramatically different signal intensity and signal-to-noise ratio

Goldstein et al. Appl Immunolistochem Mol Morphol. 2007, 15:124-33

TABLE 2. List of Factors That Could be Adjusted During the Antibody Optimization

Parameter	Description
Heating device	That is, pressure cooker, electronic water bath, microwave, steamer, hot plate
Primary antibody incubation time	This varies depending on the affinity of the antibody to its antigen target, the primary antibody concentration, incubation temperature, and antigen levels in target tissue



Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- Primary antibodies
- **Endogenous enzyme activity**
 - Effects of Avidin / Biotin
 - Detection system
 - Chromogen
 - Results interpretation

Factors Affecting IHC Staining c) Endogenous enzymes (peroxidase)

Cause	Effect	Reference
Absence of endogenous enzyme inhibition	Non-specific background staining	19, 73-76
Strong endogenous enzyme inhibition	Destruction of some antigens (i.e. CD4)	19

Bussolati G and Leonardo E. J Clin Pathol. 2008,

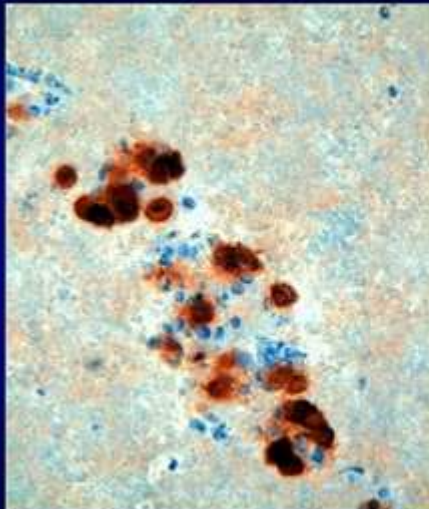


Education and Culture DG

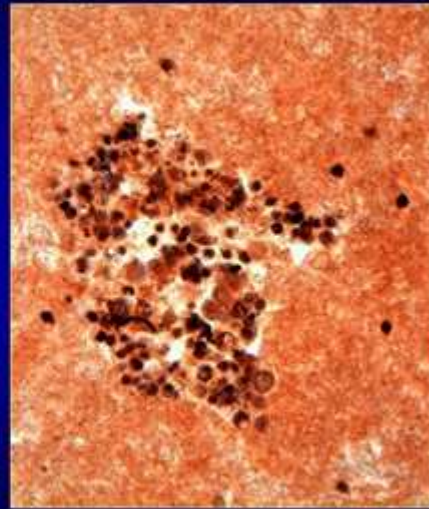
Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



Perox. Inhib.



No perox. Inhib.

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- **Effects of Avidin / Biotin**
 - Detection system
 - Chromogen
 - Results interpretation



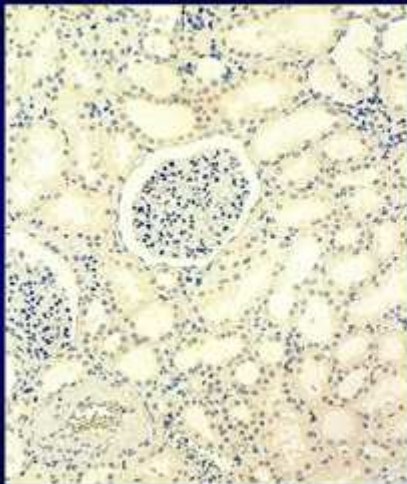
Factors Affecting IHC Staining

d) Avidin-biotin system

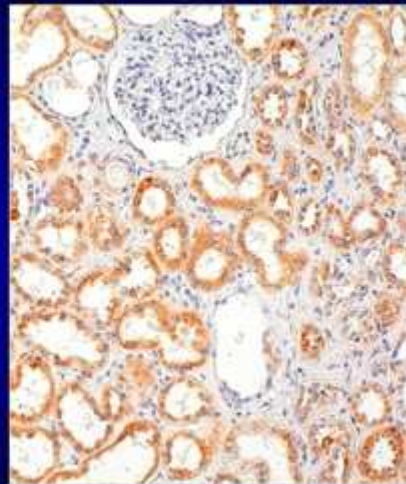
Cause	Effect	Reference
High ionic attraction of avidin	Non-immune binding to nucleic acids, phospholipids, and glycosaminoglycans	7, 78
Binding of avidin to endogenous biotin.	Strong background and false positive staining of liver, lung, spleen, adipose tissue, mammary gland, kidney, brain, gestational and post-partum endometrial cells, myelin and mast cells.	71, 79-84, 97, 98

Bussolati G and Leonardo E. J Clin Pathol. 2008, Epub ahead of print

a



b



Non-specific staining of Biotin-rich renal tubules by Avidin.

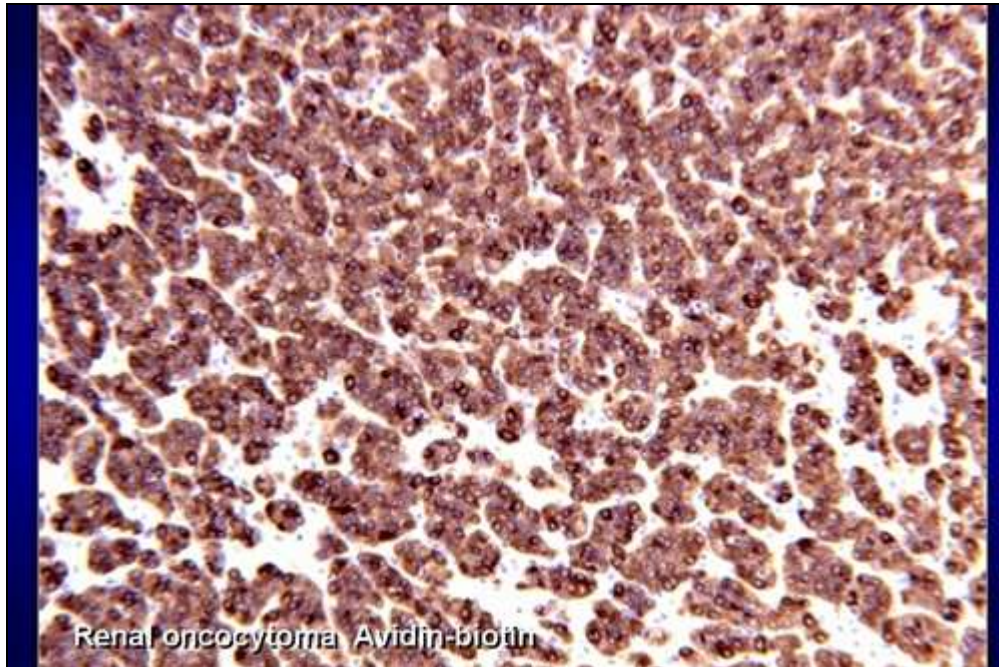


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Arch Pathol Lab Med. 1994;118:831-3.

**Anomalous immunostaining of 'optically clear' nuclei in gestational endometrium.
A potential pitfall in the diagnosis of pregnancy-related herpesvirus infection.**

Sickel JZ, di Sant'Agnese PA.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- Effects of Avidin / Biotin
- **Detection system**
 - Chromogen
 - Results interpretation

Factors Affecting IHC Staining

f) Detection system

Cause	Effect	Reference
Incorrect pH of the reaction buffer	Absence of staining	19, 110
Ionic charges of the polymers	Background staining	69, 104
Spontaneous agglutinations of the antisera	Absence of staining	69, 104

Bussolati G and Leonardo E. J Clin Pathol. 2008, Epub ahead of print



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

J Clin Pathol. 2008, 61, 1184-92

Technical pitfalls potentially affecting diagnoses in immunohistochemistry

Bussolati G, Leonardo E.

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- Effects of Avidin / Biotin
- Detection system
- **Chromogen**
- Results interpretation

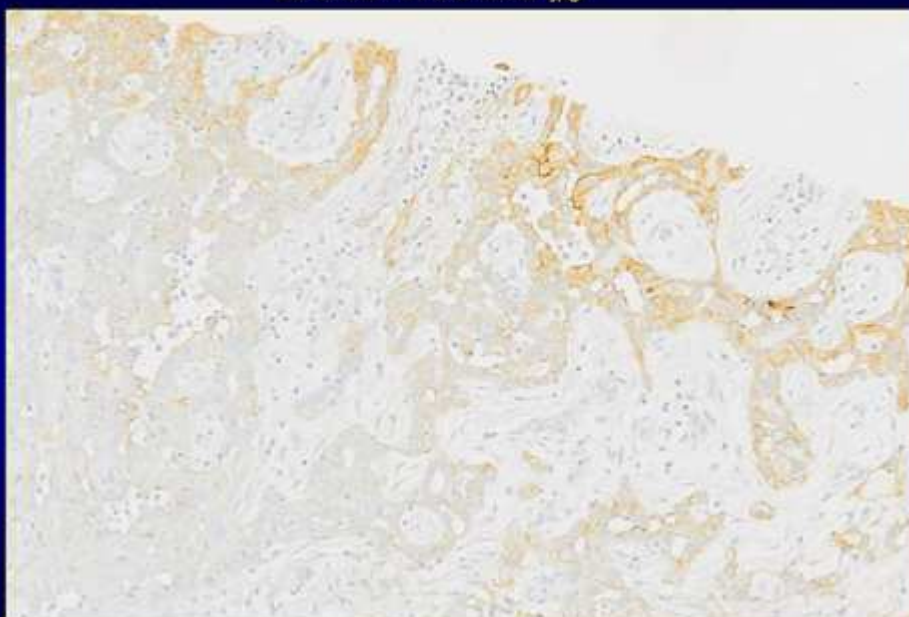


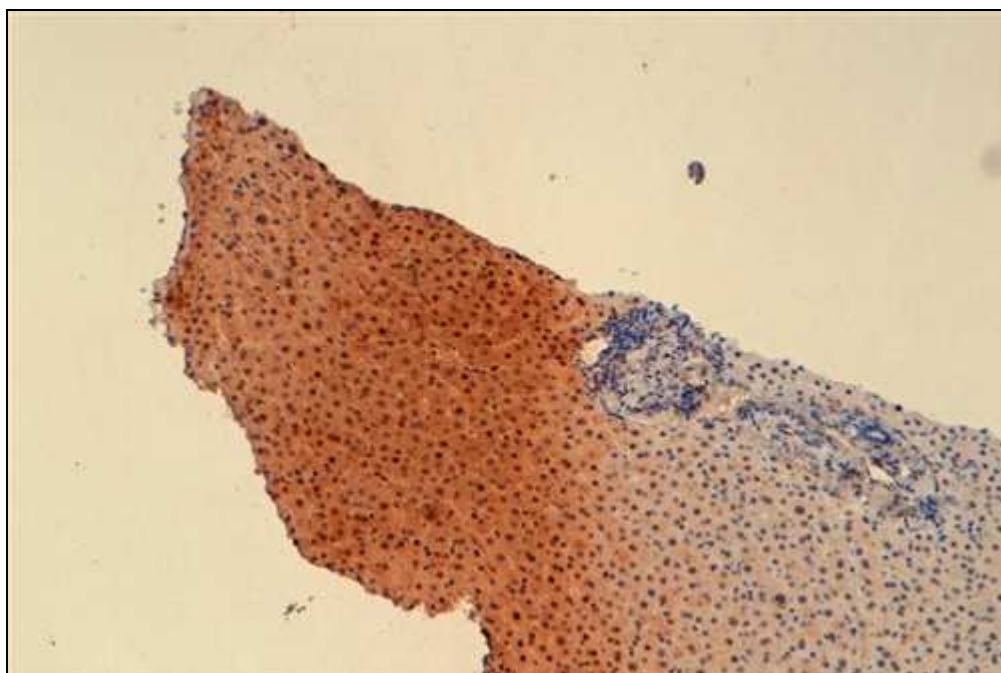
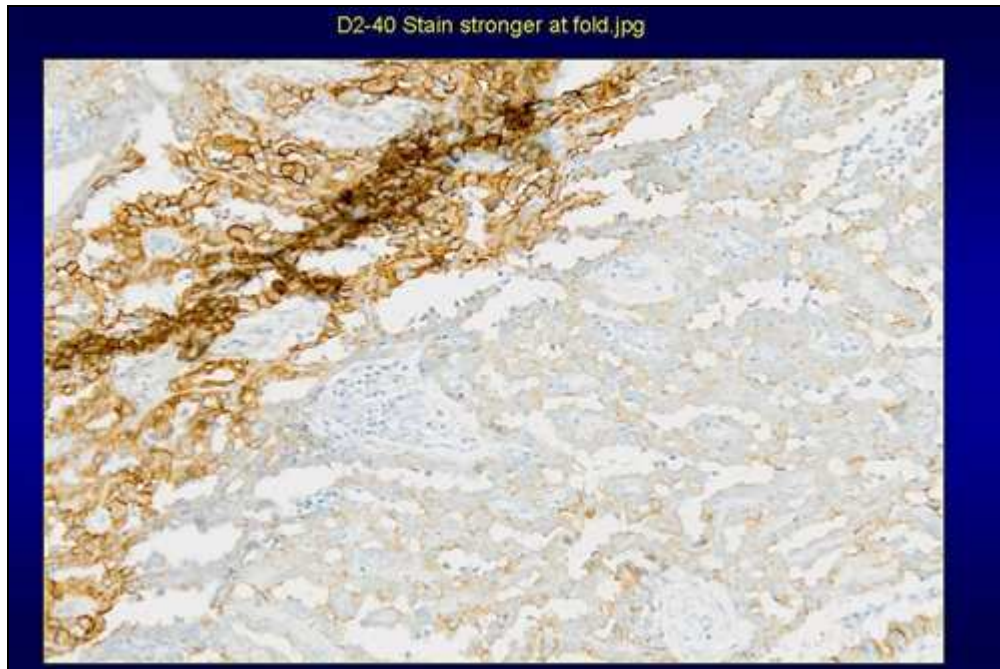
Goldstein et al. Appl Immunohistochem Mol Morphol. 2007, 15:124-33

TABLE 2. List of Factors That Could be Adjusted During the Antibody Optimization

Parameter	Description
Detection system	Polymer detection systems may allow to further dilute the antibody titer, given their generally higher sensitivity than avidin-biotin systems. Tyramine amplification systems are the most sensitive, but also most cumbersome
Chromogen	Prolonging the application of chromogen often lead to more intense signal, but could also compromise the signal-to-noise ratio

D2-40 Stain more at border.jpg







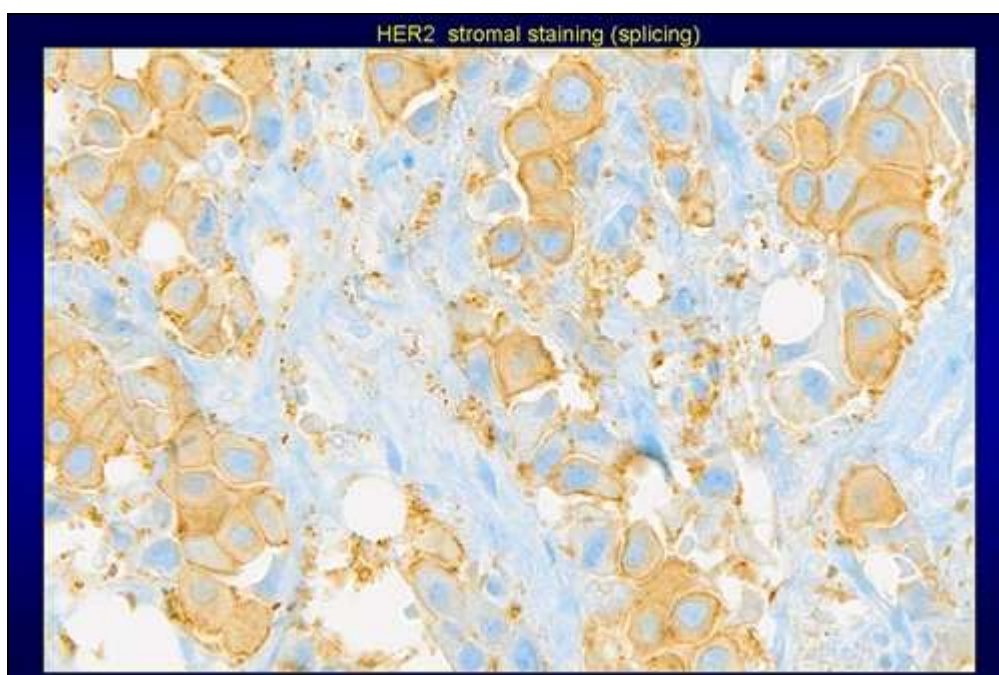
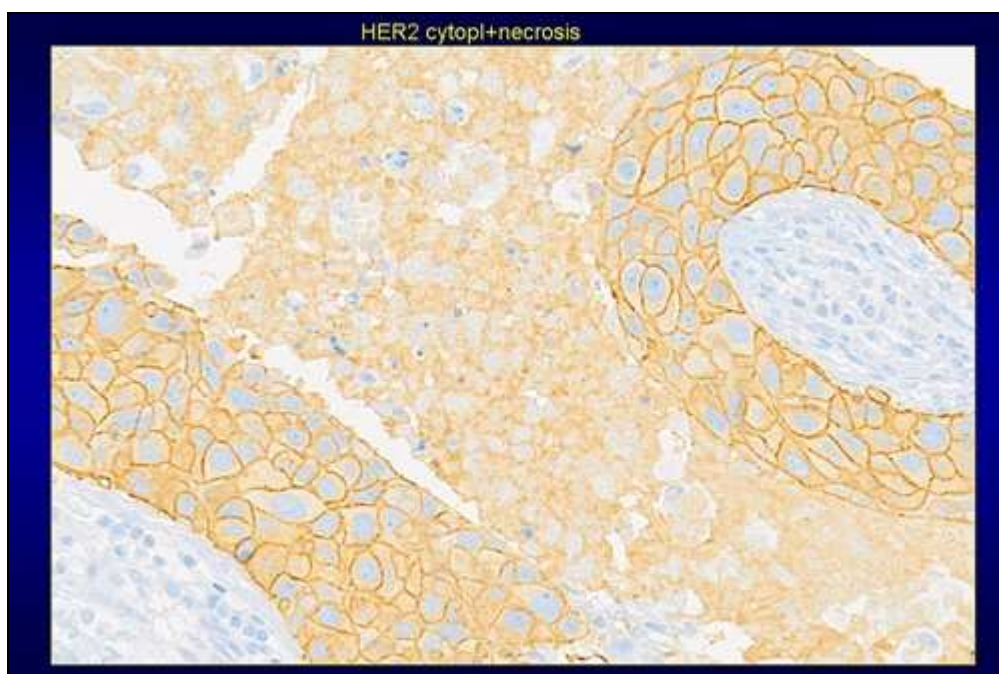
Sources of pitfalls in IHC

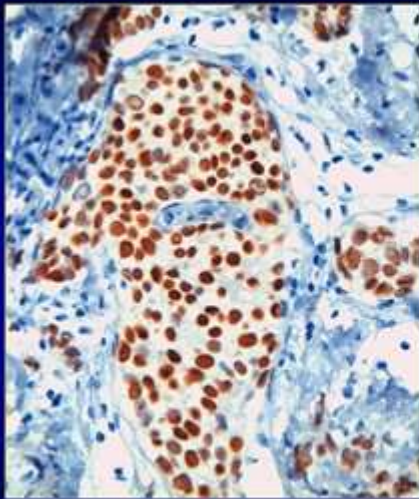
- Fixation
- Tissue processing and embedding
- Decalcification
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- Effects of Avidin / Biotin
- Detection system
- Chromogen
- **Results interpretation**

TABLE 1. IHC Assay Total Test Concept

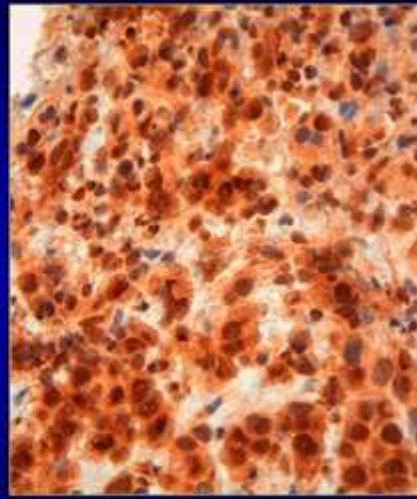
Postanalytic
Control evaluation
Results interpretation
Results reporting
Pathologist, experience and CME

From Arch Pathol Lab Med. 2000;124:945-951.





ER



ER (non-specific staining
of cytoplasm)

Appl Immunohistochem Mol Morphol. 2007;15:220-3.

**Cell membrane reactivity of MIB-1 antibody to Ki67 in
human tumors: fact or artifact?**

Leonardo E, Volante M, Barbareschi M, Cavazza A, Paolo
Dei Tos A, Bussolati G, Papotti M.

**“.... MIB-1 monoclonal antibody was also found to
stain the cell membrane of some tumor types.
..... a diagnostic feature of hyalinizing
trabecular tumor (HTT) of the thyroid.....”**



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Am J Surg Pathol. 198;8:51-5.

Immunohistochemical localization of myoglobin in nonmuscular cells.

Eusebi V, Bondi A, Rosai J.

Evaluation of result in IHC

- Semi-quantitative
- 2 criteria: - % positive cells
- Intensity of staining
- Seat of staining
- Controls:
 - Internal
 - External



Education and Culture DG

Lifelong Learning Programme



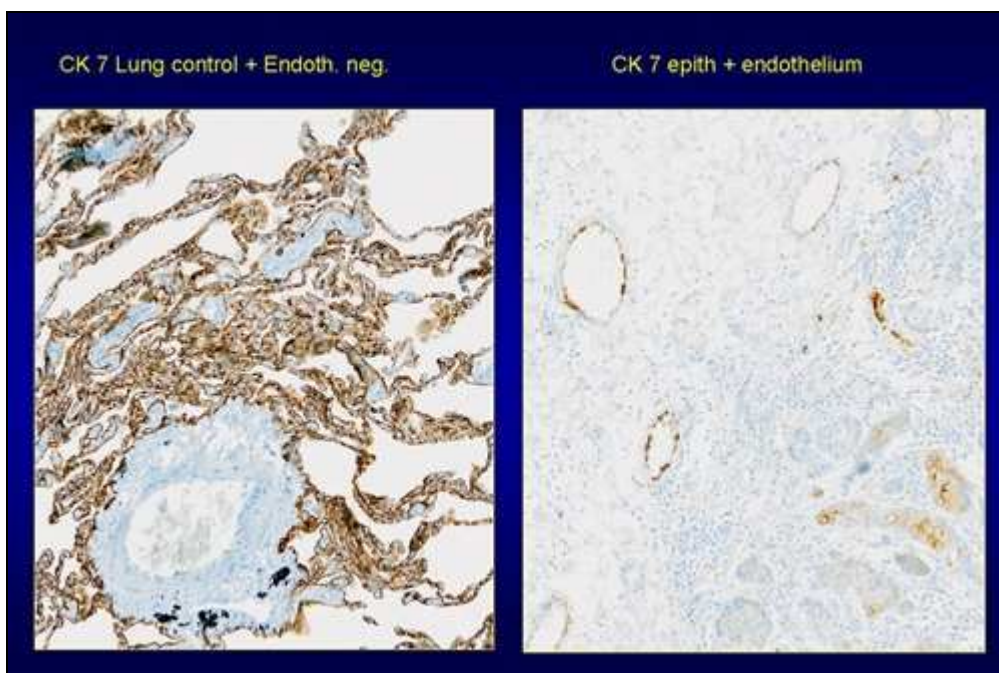
Education, Audiovisual & Culture
Executive Agency

Evaluation of IHC

- Scoring System
 - How reproducible?
 - Does it matter? (e.g. ER in breast cancer)
- Who has to do it?

Internal controls in IHC

- Surrounding tissue
- Alternative section
- Control section “added” at one side of the slide
 - e.g. appendix for S100, CK, LCA.....





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Internal controls in IHC

- Surrounding tissue
- Alternative section
- Control section “added” at one side of the slide
 - e.g. appendix for S100, CK, LCA.....
 - Tissue arrays

Use of Tissue Arrays in IHC

Virchows Arch (2006) 449:288–296
DOI 10.1007/s00428-006-0233-2

ORIGINAL ARTICLE

Routine assessment of prognostic factors in breast cancer using a multicore tissue microarray procedure

Anna Sapino • Caterina Marchiò • Rebecca Senetta •
Isabella Castellano • Luigia Macri • Paola Cassoni •
Giampiero Ghisolfi • Milena Cerrato •
Enrico D'Ambrosio • Gianni Bussolati



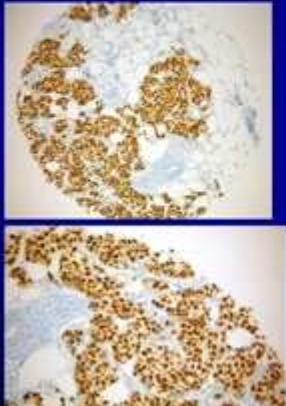
Education and Culture DG

Lifelong Learning Programme




Education, Audiovisual & Culture
Executive Agency

Dedicated Report



C-

C+



S.S.R. EGIORE FURIORE - UNIVERSITA' DI TORINO
 AZIENDA OSPEDALIERA S. GIOVANNI BATTISTA DI TORINO
 S.C.D.U. ANATOMIA PATOLOGICA II - Direttore Prof. G. Busceti
 Via Santena, 7 10126 TORINO - Tel. 011/535455 - Fax 011/535267

Tissue array N. 2010/TA/27

TISSUE ARRAY

SCHEMA:

	1	2	3	4	5	6
a						
b						
c						
d						
e						
f						

☐ ER
☐ DESM

RISULTATO

POSIZIONE	N° ISLANDI FETOLOGICI	N° FATTORI PROGNOSTICI	ER %	DES %	TA6250	HER 2	HER 2 %
1	45		10 2+	10 2+	2+	2+	14
2	4512		30 3+	80 3+	0	0	13
3	4431		95 3+	10 2+	0	0	8
4	4429		70 2+	10 2+	0	0	80
5	4430		95 2+	15 2+	2+	3+	50
6	4396		70 2+	20 2+	3+	3+	27
7	4398		2 1+	2 1+	3+	3+	30
8	4395 - 2911	4395/2911	95 2+	95 2+	0	2+	32
9							
10							
11							
12							

FATTORI DI SEZIONE INTERNA

BIOHER 4504 3+, 4512 0, 4431 0, 4429 0, 4430 2+, 4396 2+, 4398 2+, 4395 0

EQRP Sub NEO Intra-4429 2+

Torino, 20/07/2009
Firma: _____

Visto: Prof. Anna Sapino

Conclusions

- 1. Pitfalls in immunohistochemistry (IHC) may lead to incorrect interpretations and affect the diagnostic process.**
2. The main factors potentially affecting IHC staining are tissue processing and antigen retrieval procedures.
3. Important (and often neglected) issues in immunohistochemistry are type and length of tissue fixation.
4. Insidious pitfalls are represented by the presence of endogenous biotin and enzyme activity.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Conclusions

1. Pitfalls in immunohistochemistry (IHC) may lead to incorrect interpretations and affect the diagnostic process.
- 2. The main factors potentially affecting IHC staining are tissue processing and antigen retrieval procedures.**
3. Important (and often neglected) issues in immunohistochemistry are type and length of tissue fixation.
4. Insidious pitfalls are represented by the presence of endogenous biotin and enzyme activity.

Conclusions

1. Pitfalls in immunohistochemistry (IHC) may lead to incorrect interpretations and affect the diagnostic process.
2. The main factors potentially affecting IHC staining are tissue processing and antigen retrieval procedures.
- 3. Important (and often neglected) issues in immunohistochemistry are type and length of tissue fixation.**
4. Insidious pitfalls are represented by the presence of endogenous biotin and enzyme activity.



Conclusions

1. Pitfalls in immunohistochemistry (IHC) may lead to incorrect interpretations and affect the diagnostic process.
2. The main factors potentially affecting IHC staining are tissue processing and antigen retrieval procedures.
3. Important (and often neglected) issues in immunohistochemistry are type and length of tissue fixation.
- 4. Insidious pitfalls are represented by the presence of endogenous biotin and enzyme activity.**

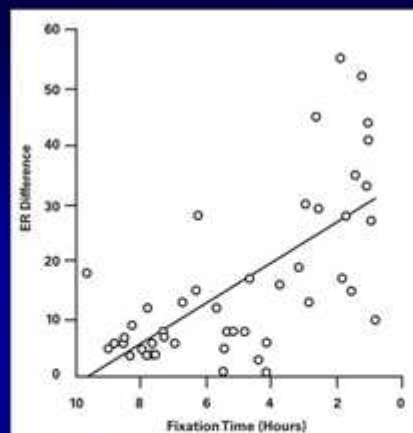


Figure 21 Needle core biopsy specimen formalin fixation time vs difference in estrogen receptor (ER) in needle core biopsy and resection specimens. The greatest disparity between ER needle core and resection specimens occurred in needle core specimens with fixation times of less than 3 hours.

Goldstein NS et al. Am J Clin Pathol. 2003;120:86-92.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Sources of pitfalls in IHC

- Fixation
- Tissue processing and embedding
- **Decalcification**
- Antigen retrieval
- Primary antibodies
- Endogenous enzyme activity
- Effects of Avidin / Biotin
- Detection system
- Chromogen
- Results interpretation

Factors Affecting IHC Staining

a2) tissue processing

Cause	Effect	Reference
Dehydration, non-polar solvents and paraffin embedding	Possible change on the conformation of some antigens	18-20
Decalcification by 10% formic acid or by 5% nitric acid	Decreased antigenicity on many antigens, particularly CD markers	21-23, 26-28

Bussolati G and Leonardo E. J Clin Pathol. 2008, Epub ahead of print



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Histopathology. 1978;2:329-34.

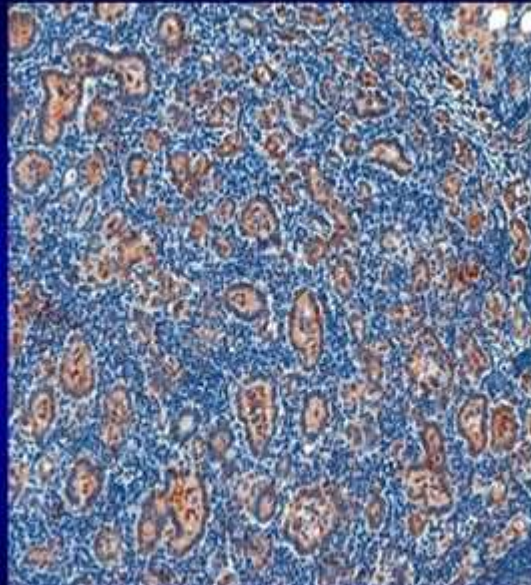
A fixation-decalcification procedure for bone biopsies.

Bussolati G

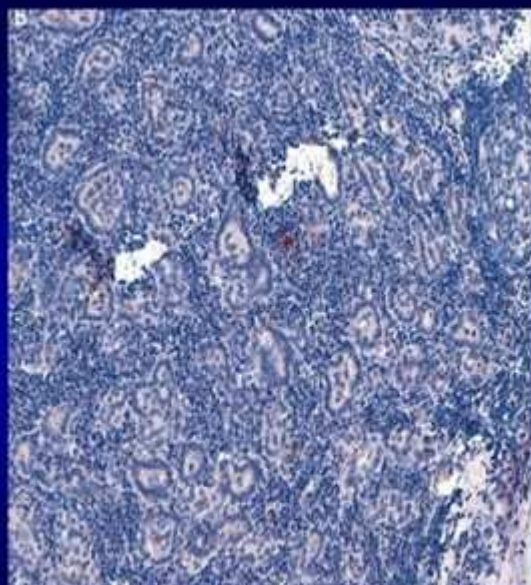
Histopathology. 2006;48:212-3

Comments to the paper: *Galectin-3 does not reliably distinguish benign from malignant thyroid neoplasms*

Bartolazzi A and Bussolati G.



Bartolazzi A and Bussolati G. Histopathology. 2006;48:212-3



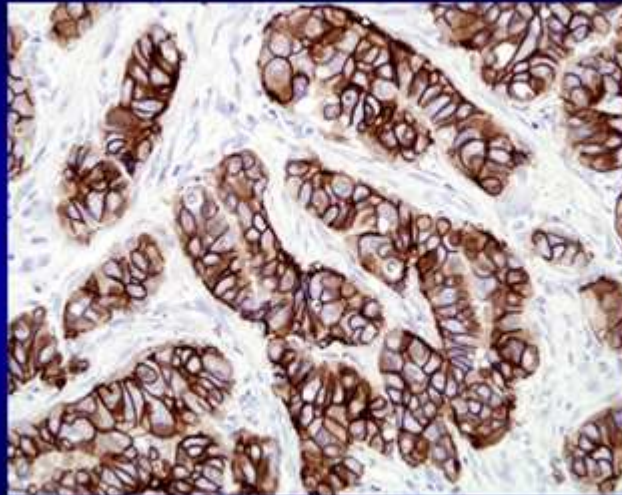
Bartolazzi A and Bussolati G. Histopathology. 2006;48:212-3



Lifelong Learning Programme



Immunohistochemistry



Protein level: How much?



Diagnostic Effusion Cytology.

Pitfalls and Algorithms.

Francesco Feoli, Dina Milowich, Christine Renard
Institut Jules Bordet, Brussels, Belgium



Summary

A 70-year-old woman with a known clinical history including malignant lymphoma, presented with a mediastinal mass. Previous pleural cytology and biopsy examinations were requested to rule out recurrent lymphoma but showed only mesothelial atypia. The cytology samples showed some artefacts. The mediastinal mass was resectable. Malignant Mesothelioma.

This case is presented to discuss the significance of atypical mesothelial proliferations, the algorithms to obtain optimal effusion cytology samples and the role of p16 testing (Hwang H and Givg A 2014).

Dr G. Sibille and Dr P. Elments respectively carried out p16-testing and the review of the original CT-Scan documents.

Clinical History.



DM Female. Born 1942.
Former Employee.

2013. Age 71. Mediastinal Mass.

1985-1994. Age 43-52.
Recurrent (x3) Abdominal Follicular Lymphoma.
Splenectomy, Radiotherapy, Chemotherapy.

2005. Age 63.
Recurrent R Pleural Effusion. R/o Lymphoma.
Talcage.

2009. Age 67.
THRSO. In Situ Carcinoma. Ascites.

2012. Age 70.
Chronic Hepatitis C.
Portal Hypertension.

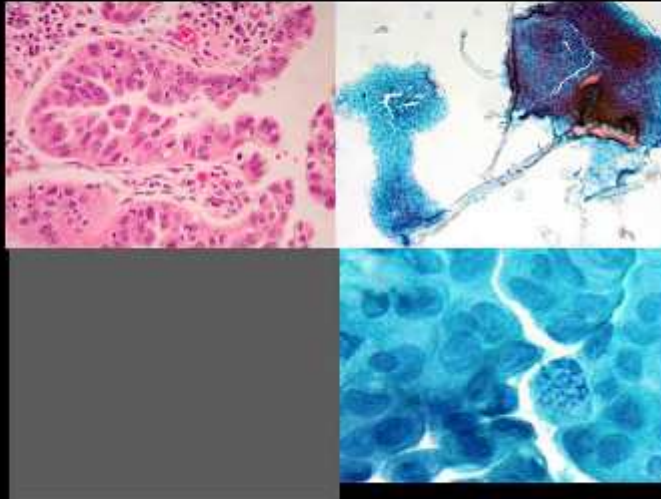


Pathology.

May 05.
Right PL Effusion
R/O Follicular Lymphoma

June 05.
Right Pleural Effusion

June 09.
T-BSC, Aortic



Effusion Cytology
Diagnostic Criteria



CYTOPATHOLOGICAL DIAGNOSIS

TWO MAJOR AREAS OF BIOLOGICAL BEHAVIOUR:

GROWTH ACTIVITY

NUCLEUS

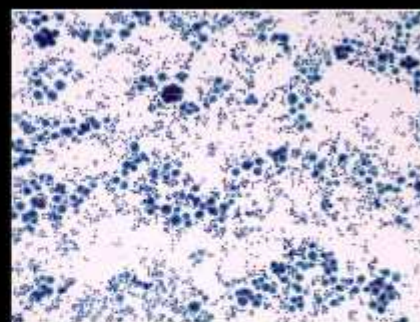


FUNCTIONAL DIFFERENTIATION.

CYTOPLASM



Cytology of The Effusions. Differential Diagnosis



BENIGN vs MALIGNANT

MESOTHELIAL vs NON MESOTHELIAL

Reactive

*Carcinoma
Other*

M. Mesothelioma

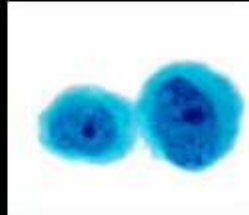


Cytology of The Effusions. Differential Diagnosis.

MESOTHELIAL vs NON MESOTHELIAL

BENIGN vs MALIGNANT

Reactive Mesothelial Cells.
Malignant Mesothelioma.



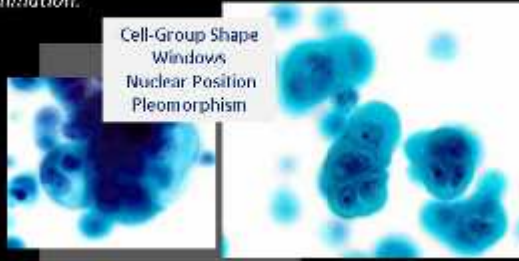
Hypercellularity
Cell Groups
Cell Size

EXTRANEOUS CELL POPULATION

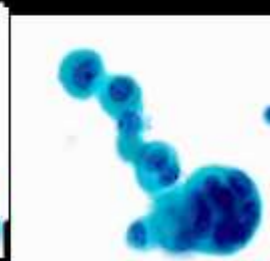
Chronic Inflammation.
Lymphoma.

Melanoma.
Sarcoma.

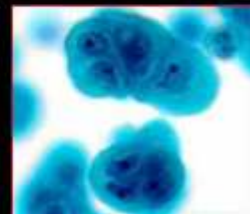
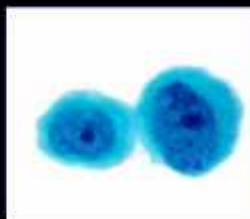
Carcinoma



Cell-Group Shape
Windows
Nuclear Position
Pleomorphism

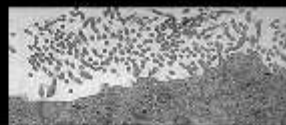


Sometimes There Is Morphological Overlap





The Many Faces of Mesothelial Cells.

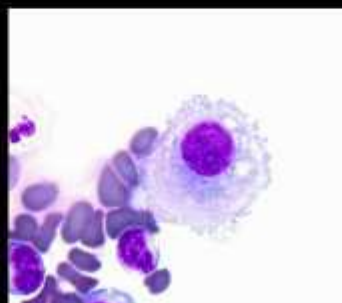
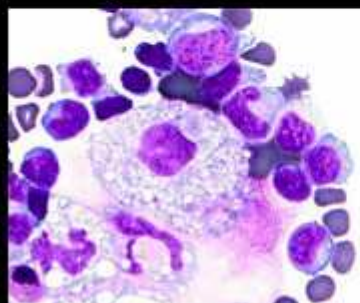


Morphologic Overlap

« The standard criteria of malignancy, based on the evaluation of single cell morphology are not applicable for most of the effusion cytology specimens. »

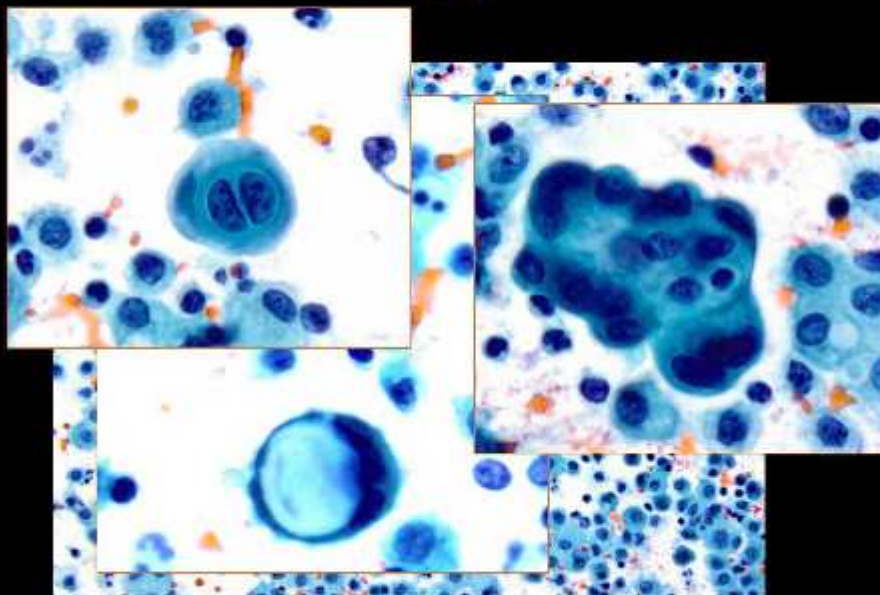
Sivdham SE & Arkinson BE 2007

The Many Faces of Mesothelial Cells. Unfixed Sample. Diff Quick Stain

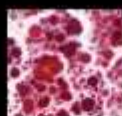




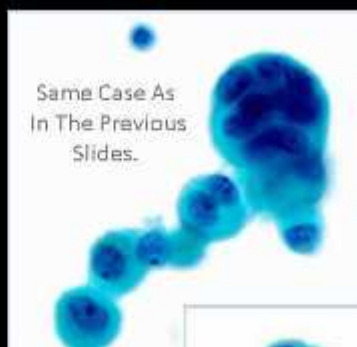
The Many Faces of Mesothelial Cells.
Unfixed Sample. Papanicolaou Cytospin.



The Many Faces of Mesothelial Cells.
Cytorich Fixed. BD-Sure Path.



Same Case As
In The Previous
Slides.




1122 (10/09/07) 1122 (10/09/07)

Table 2. Comparison of Body Cavity Fluids With Adenocarcinoma

Site	Responses, No.		Adenocarcinoma, No. (%)	
	C	IP	C	IP
Pelvic	338	203	1150 (96)	151 (90) $P < .001$
Pleural	2071	850	5961 (75)*	561 (75) $P = .92$
Pericardial	455	81	364 (80)*	61 (75) $P = .37$
Peritoneal	4855	835	3867 (79)	722 (81) $P < .001$
Total	14721	2029	11365 (77)	1642 (81)



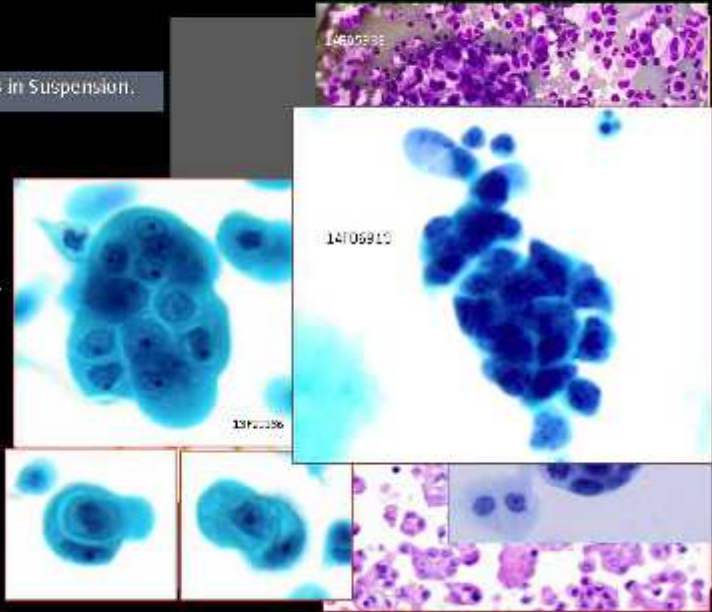
 **DIAGNOSTIC DIFFICULTIES. Morphological Overlap**


Cytoplasm. Cells in Suspension.

Architecture.

Cellularity.

Technical Quality.



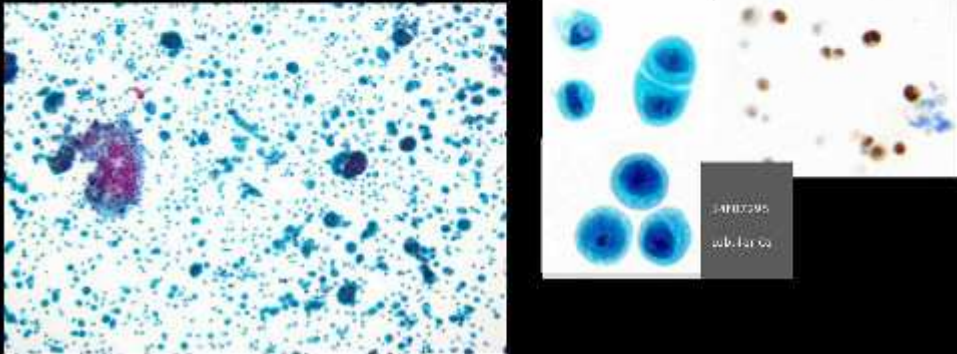
 **DIAGNOSTIC DIFFICULTIES. Morphological Overlap**

Cytoplasm. Cells in Suspension.

Architecture.

Cellularity. 2 Cell Populations.

Fluids: Good and Bad Authors
CAP from 30%
Norman AT et al. Auto Revue Feb 2004; 12(1): 140-15





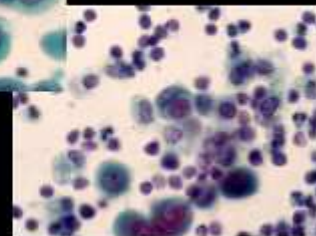
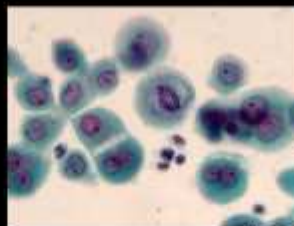
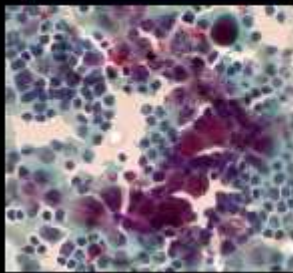
DIAGNOSTIC DIFFICULTIES. Morphological Overlap

Cytoplasm. Cells in Suspension.

Architecture.

Cellularity.

Technical Quality.



2013: The Solution.
Metastatic Malignant Mesothelioma.

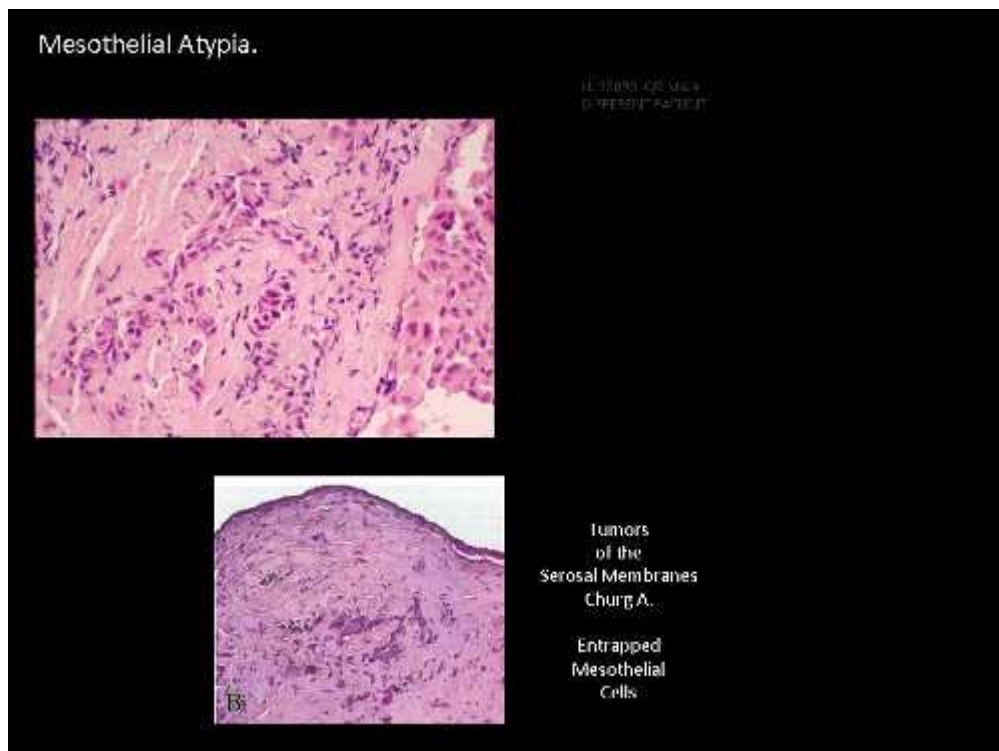
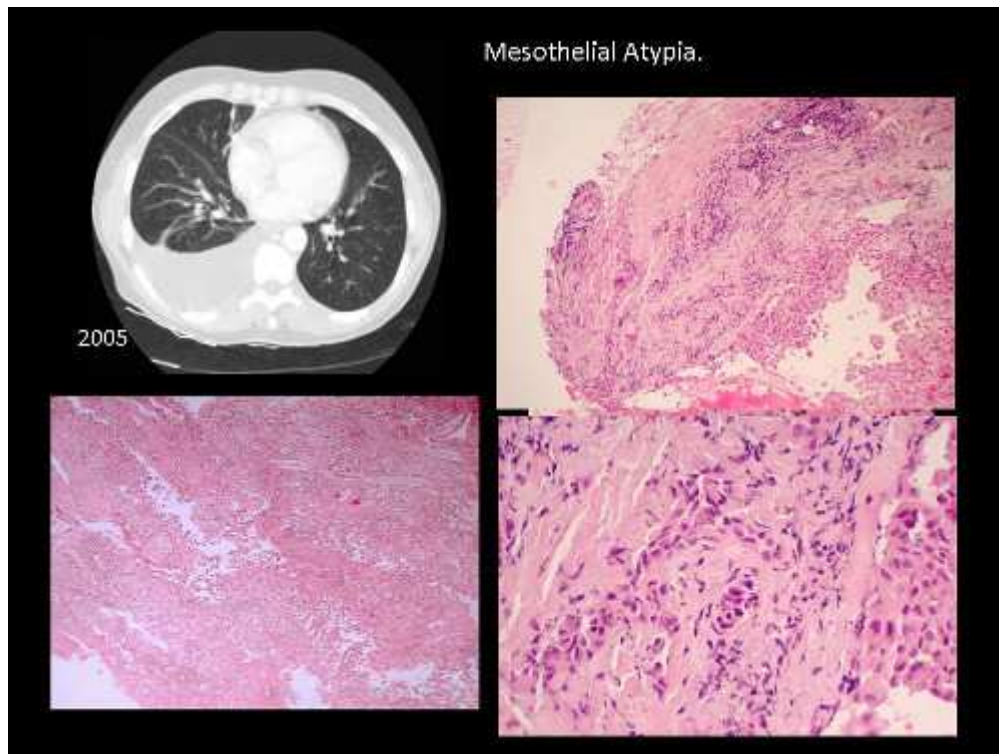


2013

Differential

Mesothelioma: mesothelial cells involving the parietal and the mediastinal lymph nodes. Almost always associated with chronic effusion or sarcomatous. *Virch Pathol* 1995; 29:333-346

Unusual features of malignant mesothelioma metastatic to the mediastinal lymph nodes. *Appl Immunohistochem Mol Morphol* 2008; 16:392-7





Pitfall? Clinical Evolution : 2005-2013.

CYTOTOLOGY
Sensitivity: 95%-97% Cytosins, C. B. cells, B. Multiple Biopsy + 20%-35%
Specificity: 95%
Complete Sensitivity for Mesothelioma: 32%

Low PPV for M. Mesothelioma.

R/o Lymphoma.

Chemotherapy.

Thoracoscopic Description Non Available.

No Unconcentrated Cytologic Sample.

Drying Artefacts & Overstaining.

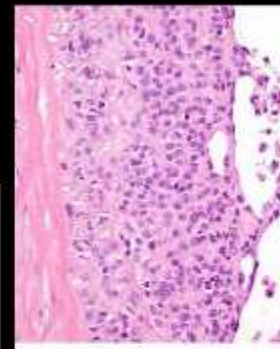
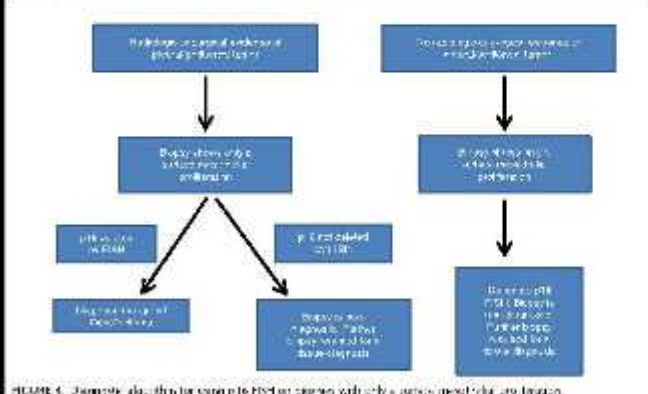
No Cell Block.

p16 FISH Deletion in Surface Epithelial Mesothelial Proliferations Is Predictive of Underlying Invasive Mesothelioma


Huay-Huang, MD,¹ Christopher T. The, MD,^{2,3} Stephanie Rodriguez, HT, ASCP,⁴
Arina Garcia, MD,² and Andrew C. Goss, MD¹

Am J Surg Pathol • Volume 38, Number 5, May 2014

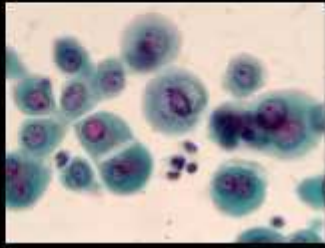
DOI: 10.1097/PAF.0000000000000000

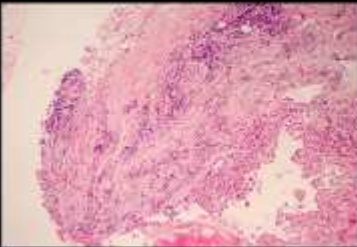






Our Patient
2005.





FISH 2014, 9p21

88%	:	2	:	2	•
8%	:	3	:	3	• ←
2%	:	1	:	1	•
2%	:	3	:	2	• ←

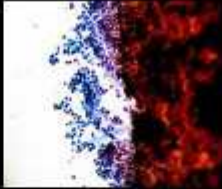


Algorithms.

Prefer Fresh Effusions.
If Needed: Refrigerate 4°C (2 Weeks or Longer)

Heparin 3U/mL fluid, (May Interfere with Diff Quick)
EDTA.

Possible: 1:1 Fixation
(Before Cytopreparation. CytoLys, CytoRich, Ethanol, etc.)

1% Saponin/ 3% Ca Gluconate Hemolysis.

Direct Fresh Smear Diff.Quick. Unconcentrated: Sample 3 Drops + Albumin 22% 1 Drop.

Diff Quick Cytospin.

Wet Fixed Pap: Glucopap Sure Path, ThinPrep

Cell Blocks 2% Agar & 10% Formalin: Reserve for Additional Samples, or Histology.