Dal prelievo all'analisi molecolare: certezze e criticità

La gestione del tessuto

Isabella Castellano
Anna Sapino

Dipartimento di Scienze Mediche
Università degli Studi di Torino
Città della Salute e della Scienza di Torino
Anatomia Patologica 2
IRCCS Candiolo, Torino

Il cambiamento in anatomia patologica:

DA…..

classificazione delle malattie ***esclusivamente*** dal punto di vista morfologico

A,,,,,

definizione di biomarcatori, di alterazioni geniche o di marcatori presenti nei tessuti patologici, che possono essere indispensabili per l'accurata impostazione di terapie mirate a bersaglio molecolare (target therapy).

La **corretta gestione** del tessuto diventa prioritaria per garantire una diagnosi corretta e completa, ma anche **la sua custodia nel tempo** per assolvere ad eventuali richieste del paziente che necessita del preparato per ulteriori analisi o per altre esigenze cliniche.

Standardizzare le procedure per…. le necessità dei pazienti

A recent “viewpoint” article [Goetz L et al JAMA 2013] calls on pathologists to consider that as **genomic testing** becomes part of routine care and patients become increasingly informed, **the workflow of pathology lab will have to adapt to meet the demands of the “next generation of patients”**.

- GEP
- Mutational Analysis
- Xenopatient model (viable tissue)

Standardizzare per….
le necessità della ricerca

- Dr. Compton, Director of the National Cancer Inst. Office of Biorepositories and Biospecimen Research

"*billions of dollars have been wasted in the past because researchers developing biomarkers supposed to be predictive of cancer and responses to therapies relied on tissue samples that were utterly useless: tissue had been subjected to careless handling and storage and sampling procedure were missing, so that results were not reproducible*".

Ministero della Salute

Dedicated Working Group on:

1. Tracking
2. **Collection**
3. **Transfer**
4. **Preservation**
5. Archival

… of tissues and cells
for diagnostic analyses

Anatomia Patologica: procedure pre-analitiche (tutto ciò che succede prima diagnosi al microscopio)

Surgical Theater

Pathology Lab

Grossing

Fixation

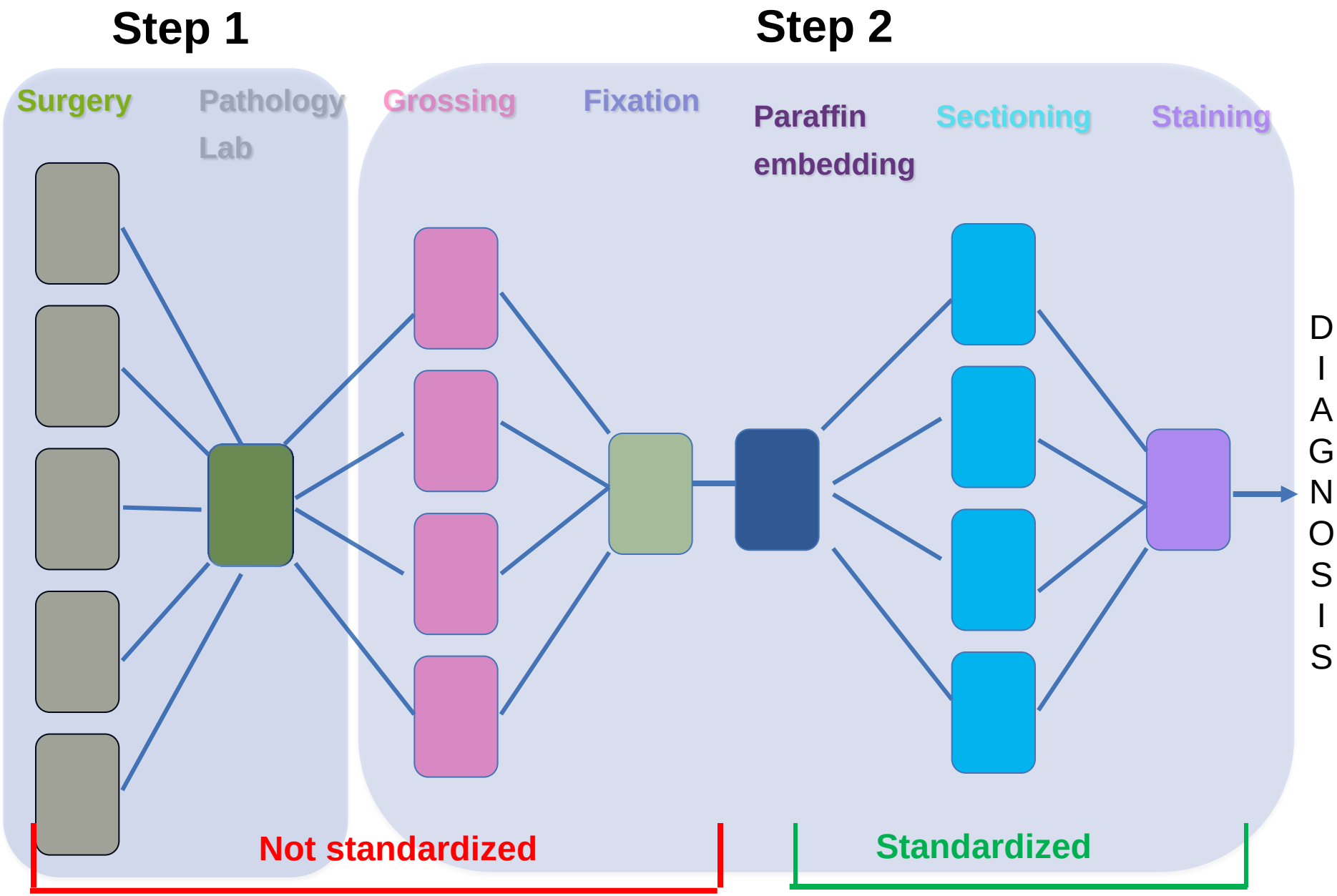
Processing

Paraffin embedding

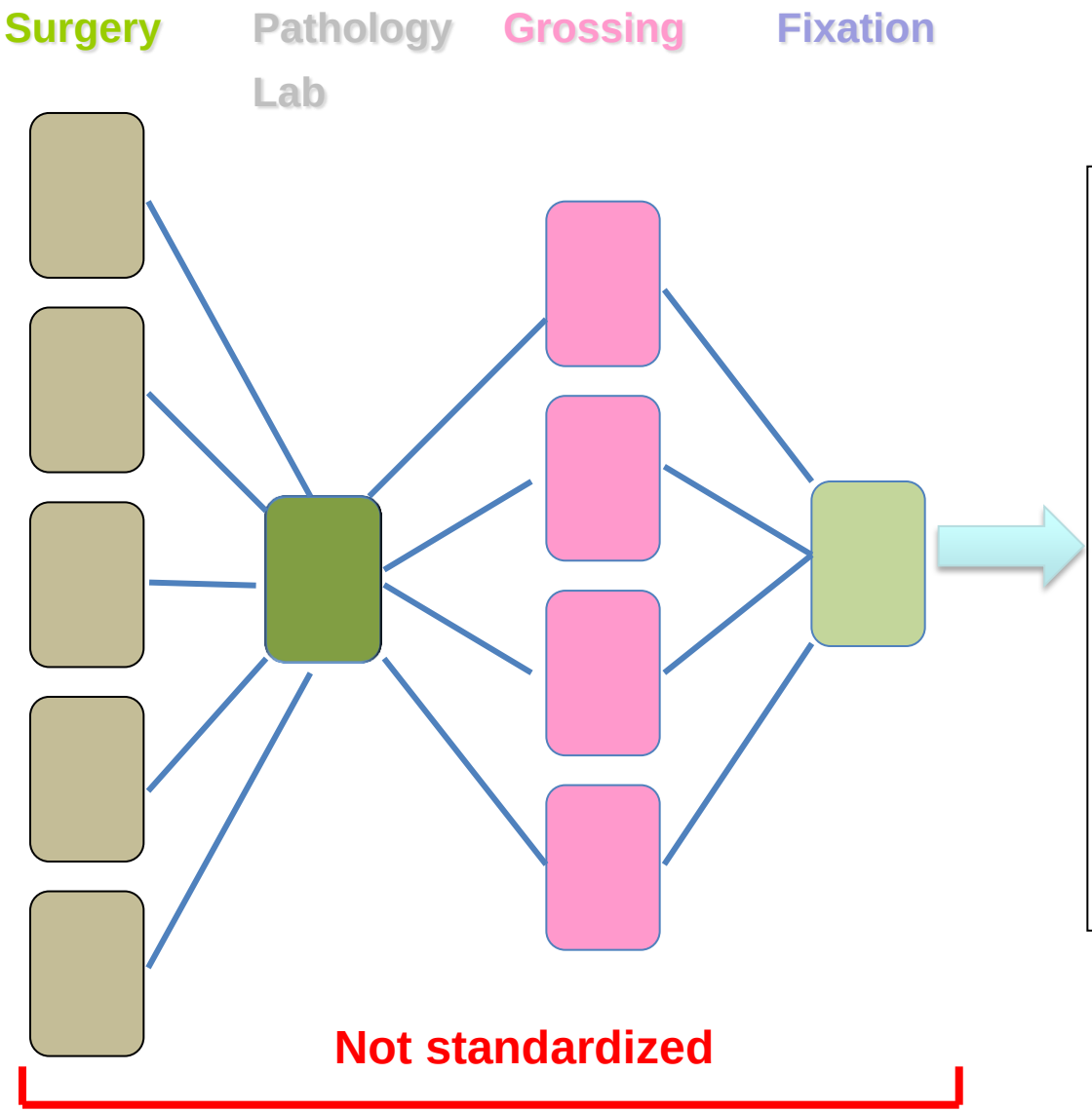
Sectioning

Staining

Anatomia Patologica: procedure pre-analitiche



Anatomia Patologica: procedure pre-analitiche



- **Structure** (morphological diagnosis)
- **Proteins** (Immuno-histochemistry)
- **Nucleic Acids** (FISH, Mutation, GEP)

VARIBILI PRE-ANALITICHE CHE INFLUENZANO I DATI ISTOPATOLOGICI E MOLECOLARI

- VARIABILE «UMANA»
- TEMPO
- TEMPERATURA
- FISSAZIONE

VARIBILI PRE-ANALITICHE CHE INFLUENZANO I DATI ISTOPATOLOGICI E MOLECOLARI

- **VARIABILE «UMANA»**
- TEMPO
- TEMPERATURA
- FISSAZIONE

Pre-analytical procedures affected by human activities

Surgical Theater

Pathology Lab

Grossing

Fixation

Processing

Paraffin embedding

Sectioning

Staining

VARIBILI PRE-ANALITICHE CHE INFLUENZANO I DATI ISTOPATOLOGICI E MOLECOLARI

- **VARIABILE «UMANA»**
- **TEMPO**
- **TEMPERATURA**
- FISSAZIONE

FASE PRE-ANALITICA DELLE PROCEDURE ISTOLOGICHE

SALA OPERATORIA

ANATOMIA PATOLOGICA

Step 1

WARM ISCHEMIA

COLD ISCHEMIA

ESAME MACROSCOPICO

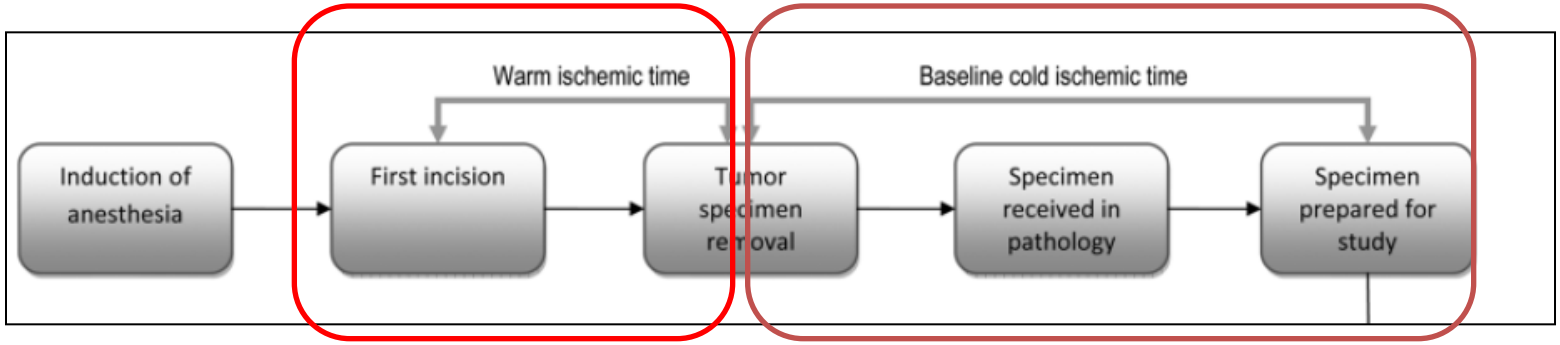
FISSAZIONE

INCLUSIONE IN PARAFFINA

TAGLIO

COLORAZIONE

Step 2



JNCI, Vol. 103, Issue 24 | December 21, 2011

Warm Ischemic Time:

Intervallo tra la legatura dell'arteria e rimozione del tessuto dal paziente.
Può variare in base a:
Complessità/modalità dell'intervento
Abilità del chirurgo
Il tessuto è vivo, reattivo e subisce I processi metabolici legati all'ipossia

Cold Ischemic Time:

Intervallo tra la rimozione del tessuto dal paziente e l'incisione per la sua adeguata fissazione

The effects of timing of fine needle aspiration biopsies on gene expression profiles in breast cancers

Vietty Wong[†1], Dong-Yu Wang[†1], Keisha Warren[1], Supriya Kulkarni[2], Scott Boerner[3], Susan Jane Done[1,3,4,5] and Wey Liang Leong[*1,6,7]

BMC Cancer. 2008 Sep 30;8:277.

Influence of Surgical Manipulation on Prostate Gene Expression: Implications for Molecular Correlates of Treatment Effects and Disease Prognosis

Daniel W. Lin, Ilsa M. Coleman, Sarah Hawley, Chung Y. Huang, Ruth Dumpit, David Gifford, Philip Kezele, Hau Hung, Beatrice S. Knudsen, Alan R. Kristal, and Peter S. Nelson

J Clin Oncol. 2006 Aug 10;24(23):3763-70.

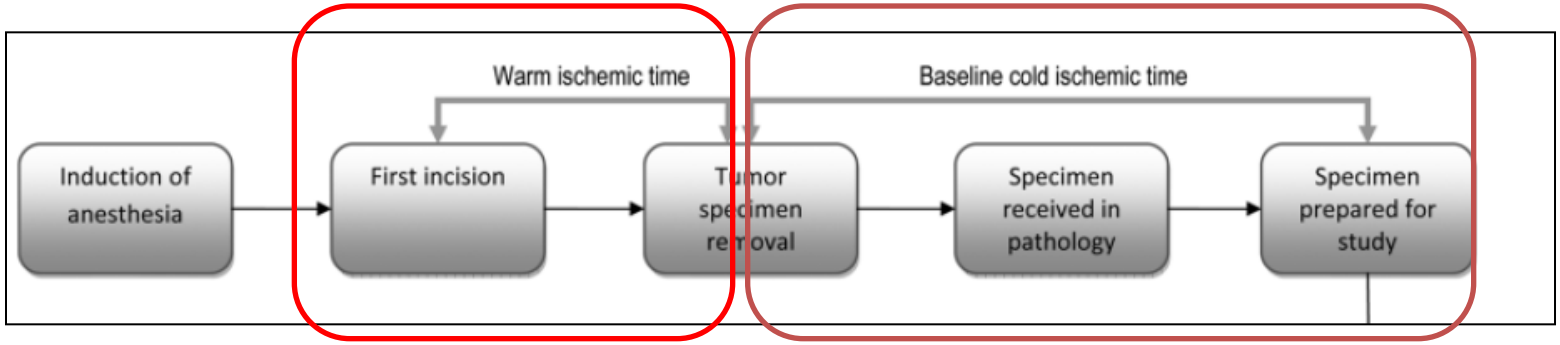
Esistono geni differenzialmente espressi prima e dopo il trattamento chirurgico

Surgical pathology: pre-analytical procedures

Surgical Theater

TIME OF SURGICAL PROCEDURE (warm ischemia time)

- As short as possible
- Pre-operative FNA to be considered



JNCI, Vol. 103, Issue 24 | December 21, 2011

Warm Ischemic Time:

Intervallo tra la legatura dell'arteria e rimozione del tessuto dal paziente.
Può variare in base a:
Complessità/modalità dell'intervento
Abilità del chirurgo
Il tessuto è vivo, reattivo e subisce I processi metabolici legati all'ipossia

Cold Ischemic Time:

Intervallo tra la rimozione del tessuto dal paziente e l'incisione per la sua adeguata fissazione

Tempo di trasporto: cold ischemia time

Intervallo dall'escissione chirurgica del pezzo alla sua fissazione.

Dipende da:

- Organizzazione delle sale operatorie
- Localizzazione del laboratorio di anatomia patologica

Histopathology 2010, **56**, 240–250. DOI: 10.1111/j.1365-2559.2009.03470.x

Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,[1] David A Levison,[1] Robert J C Steele & Neil M Kernohan[1]
Department of Surgery & Molecular Oncology, and [1]Department of Pathology & Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK

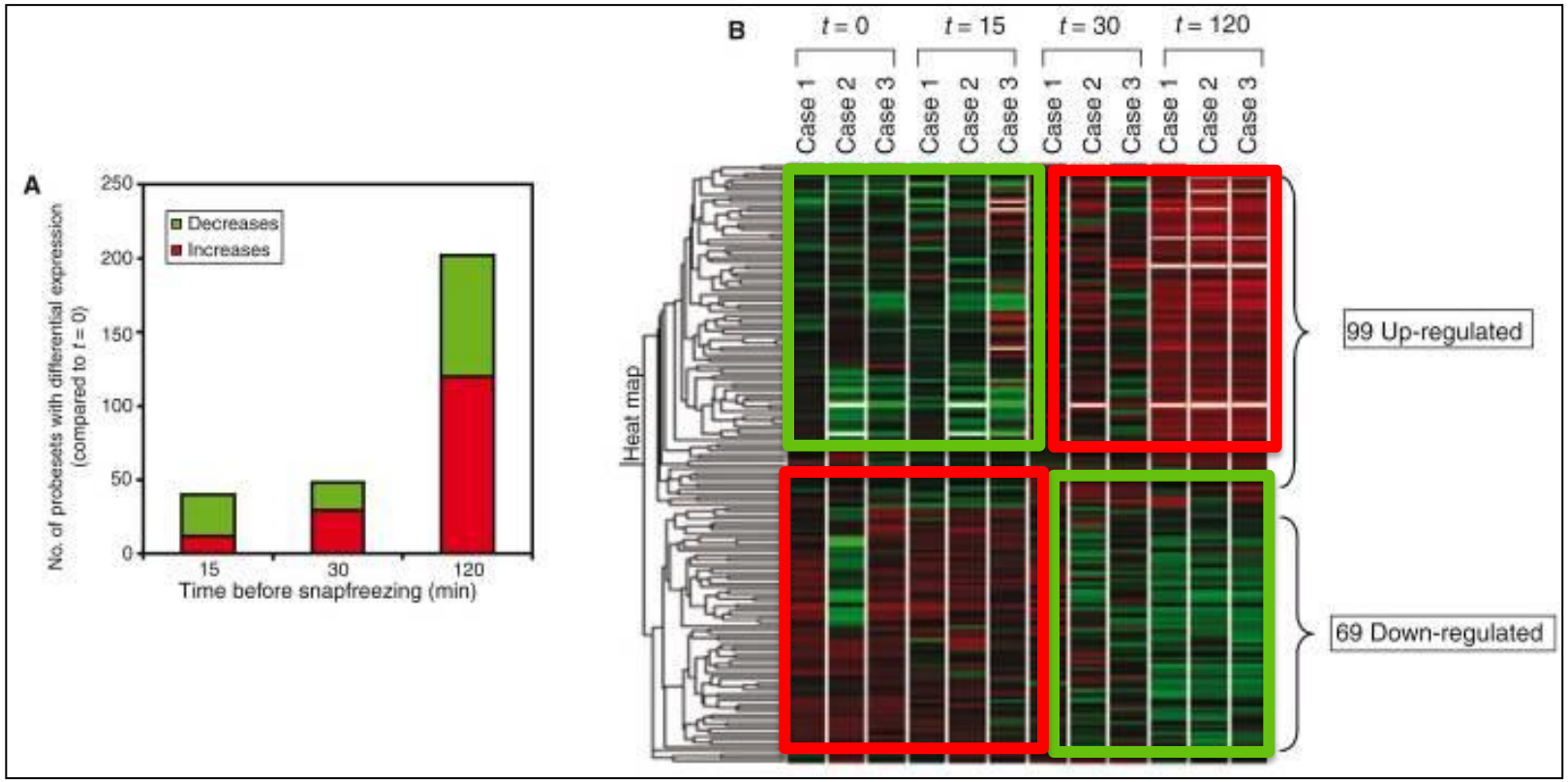
Esiste una variazione (riduzione o aumento) del livello di espressione genica già dopo **15 minuti**.
Dopo **120 minuti** il livello di espressione di certi geni può anche variare del doppio

La riduzione di espressione di KLF6 è implicata nella patogenesi di circa 1/3 dei tumori. Ma la cold ischemia può favore un significativo aumento della sua espressione, fornendo un dato non attendibile.

Histopathology 2010

Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol

Susan E Bray, Fiona E M Paulin, Siew Chinn Fong, Lee Baker, Frank A Carey,[1]
David A Levison,[1] Robert J C Steele & Neil M Kernohan[1]



It is clear that **unless detailed tissue-handling protocols are in place**, misleading results may be obtained.

Stability of Phosphoprotein as a Biological Marker of Tumor Signaling

Amanda F. Baker,[1] Tomislav Dragovich,[1] Nathan T. Ihle,[1] Ryan Williams,[1]
Cecilia Fenoglio-Preiser,[2] and Garth Powis[1]

Clin Cancer Res 2005

Temperature:RT

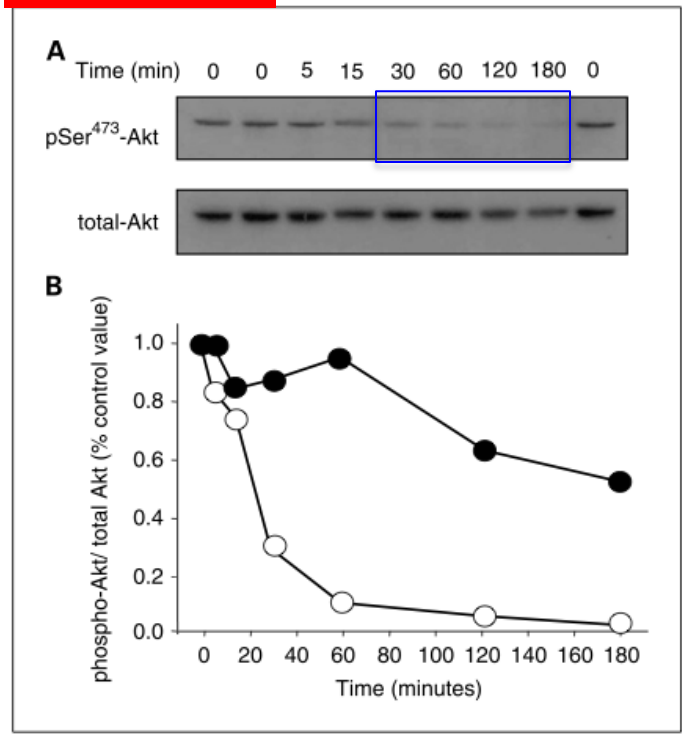


Fig. 2. Stability of phospho-Akt in HT-29 human colon cancer xenografts. HT-29 human tumor xenografts were excised from *scid* mice and kept at room temperature for the times shown. Small pieces were then rapidly frozen in liquid N_2 for Western blotting. *A*, phospho-Ser^{473}-Akt and total Akt measured by Western blotting. *B*, time course of the loss of total Akt (●) and phospho-Ser^{473}-Akt (○) relative to total-Akt.

Results IHC: p-Akt staining was present in fresh sample but not in tissue that had
been allowed to stand for 30 minutes at room temperature.

Cold ischemia time and immunohistochemistry

Anatomic Pathology / Effect of 96-Hour Formalin Fixation on ER/PR/HER2

The Effect of 96-Hour Formalin Fixation on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Carcinoma

Isil Z. Yildiz-Aktas, MD,[1] David J. Dabbs, MD,[1] Kristine L. Cooper, MS,[2] Mamatha Chivukula, MD,[1] Kim McManus, HT(ASCP),[1] and Rohit Bhargava, MD[1]

The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma

Isil Z Yildiz-Aktas, David J Dabbs and Rohit Bhargava

Anatomic Pathology / Fixation Effects on ER and PR in Breast Cancer

Delay to formalin fixation 'cold ischemia time': effect on ERBB2 detection by *in-situ* hybridization and immunohistochemistry

Bryce P Portier[1], Zhen Wang[1], Erinn Downs-Kelly[1], Jordi J Rowe[1], Deepa Patil[1], Chis Lanigan[1], G Thomas Budd[2], David G Hicks[3], David L Rimm[4] and Raymond R Tubbs[1]

The Effect of Delay in Fixation, Different Fixatives, and Duration of Fixation in Estrogen and Progesterone Receptor Results in Breast Carcinoma

Sophia Apple, MD, MS, Richard Pucci, Alarice C. Lowe, MD, Itsushi Shintaku, PhD, Saeedeh Shapourifar-Tehrani, MPH, and Neda Moatamed, MD

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

Clinical Study

The Effect of Cold Ischemia Time and/or Formalin Fixation on Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor-2 Results in Breast Carcinoma

Melike Pekmezci,[1] Anna Szpaderska,[2] Clodia Osipo,[1] and Çağatay Erşahin[1]

Modern Pathology (2012), 1–8

The effect of prolonged cold ischemia time on estrogen receptor immunohistochemistry in breast cancer

Xiaoxian Li[1], Michael T Deavers[1], Ming Guo[1], Ping Liu[2], Yun Gong[1], Constance T Albarracin[1], Lavinia P Middleton[1] and Lei Huo[1]

Breast Cancer Predictive Factor Testing: The Challenges and Importance of Standardizing Tissue Handling

David G. Hicks, LeeAnn Kushner, Kristin McCarthy

J Natl Cancer Inst Monogr 2011;42:43–45

Cold Ischemic Time and the Potential Impact on Breast Predictive Factor Testing

ERs and progesterone receptor (PR) are thermolabile proteins whose levels of expression are altered by prolonged cold ischemic time (10). Recent reports suggest that delays from tissue collection to the initiation of formalin fixation may adversely affect both hormone receptor assays (10,11) and HER2 analysis (11) and that some tumors with excessive cold ischemic times may be falsely classified as negative. The consequences of invalid breast predictive factor testing could be catastrophic to the patient and has the potential to change the type of adjuvant therapeutic regimen offered, which in turn could adversely affect outcome.

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

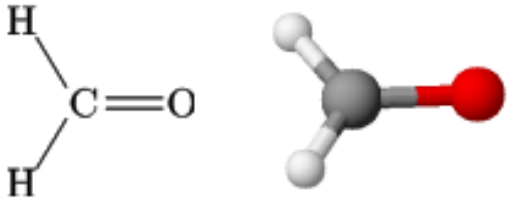
Preanalytic standardization:

- *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 to1 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

Metodi di trasporto

- A «FRESCO»: gold standard
- IN FORMALINA
- UNDER VACUUM E CONSERVATI A 4° C



FORMALDEIDE DEFINITA CANCEROGENA

livello 1/b dal 1 gennaio 2016:

IL SUO UTILIZZO E' PROIBITO NEI LUOGHI DOVE NON ESISTONO DEI PRESIDI DI PROTEZIONE

(CAPPE BIOLOGICHE ASPIRANTI)

Regolamento (UE) 2015/491 del 23 marzo 2015

Sci Total Environ. 2010 Jul 15;408(16):3092-5. Epub 2010 May 4.

Vacuum-based preservation of surgical specimens: an environmentally-safe step towards a formalin-free hospital.

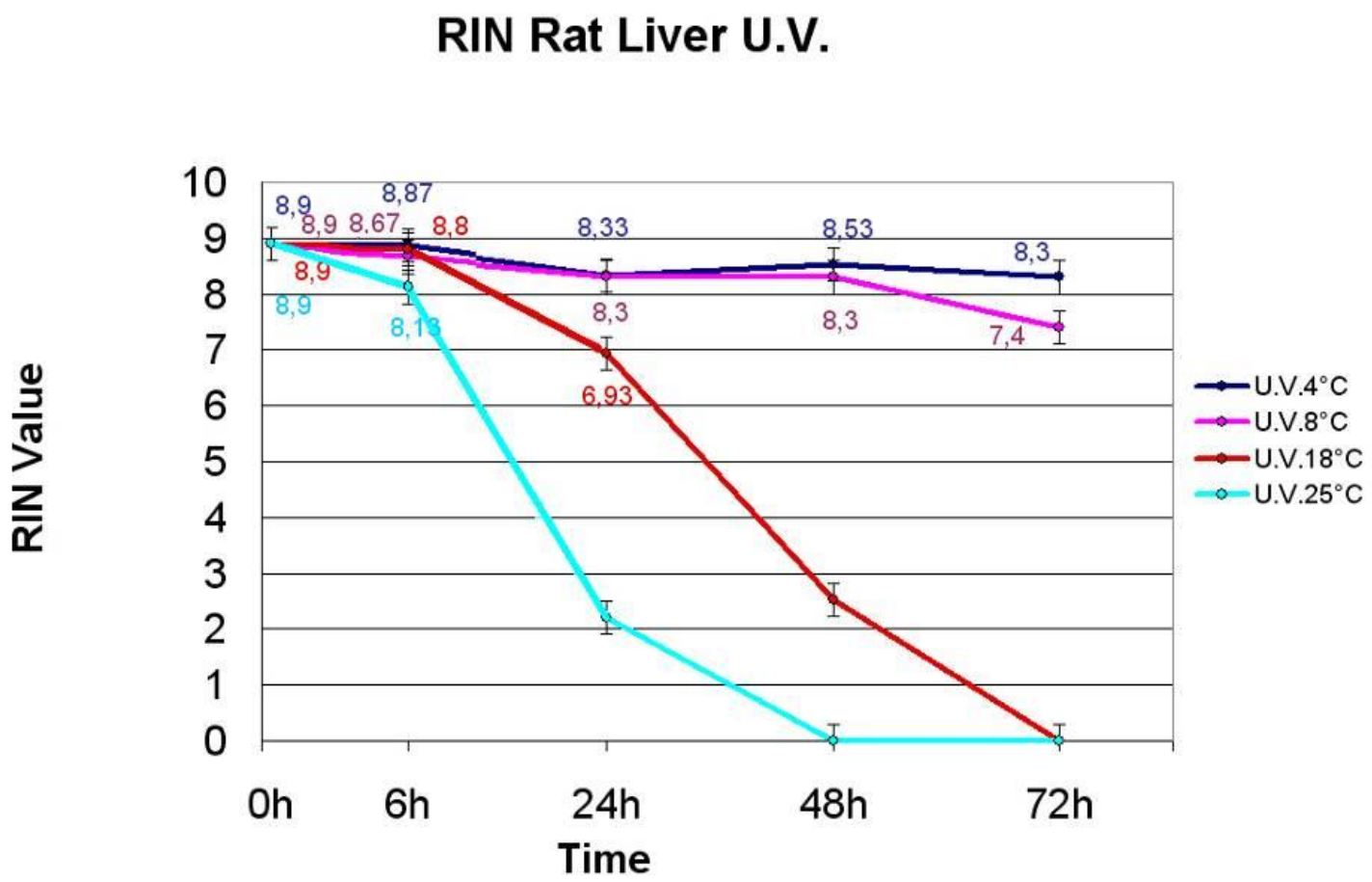
Di Novi C, Minniti D, Barbaro S, Zampirolo MG, Cimino A, Bussolati G.
Dept. Of Public Policy and Choice University of Eastern Piedmont, Italy.

Tessuti messi in un sacchetto di plastica sottovuoto:

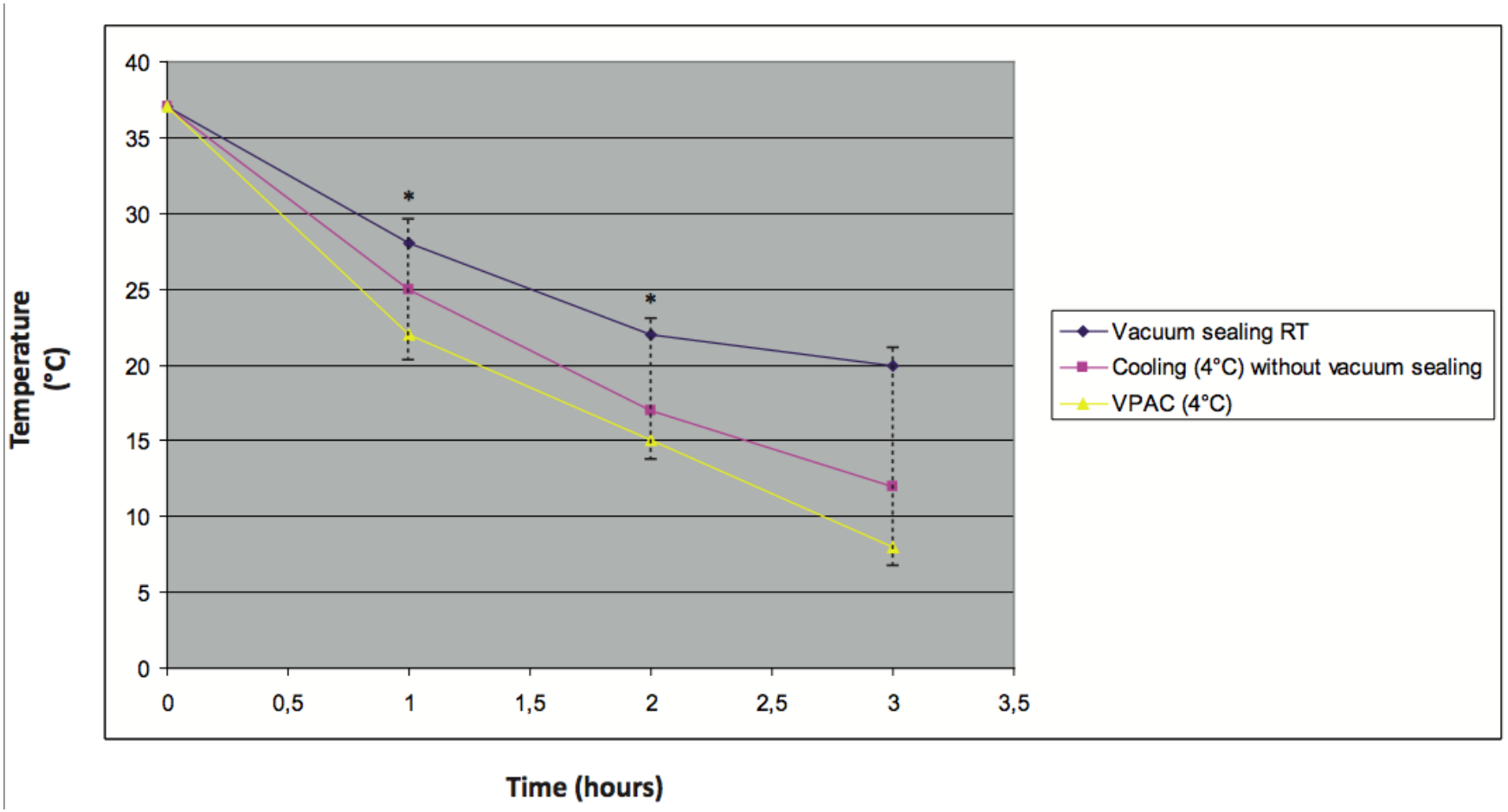
- NO essiccamento
- favorisce il raffreddamento
- facilita il trasporto

Conservazione a 4°C, sino al trasferimento in Anatomia Patologica, per un tempo variabile tra poche ore fino a 72 ore.

Under vacuum sealing for transport of surgical specimens



Temperature decrease over time: Vacuum packing + cooling = optimal



Annaratone et al, PloOne 2013

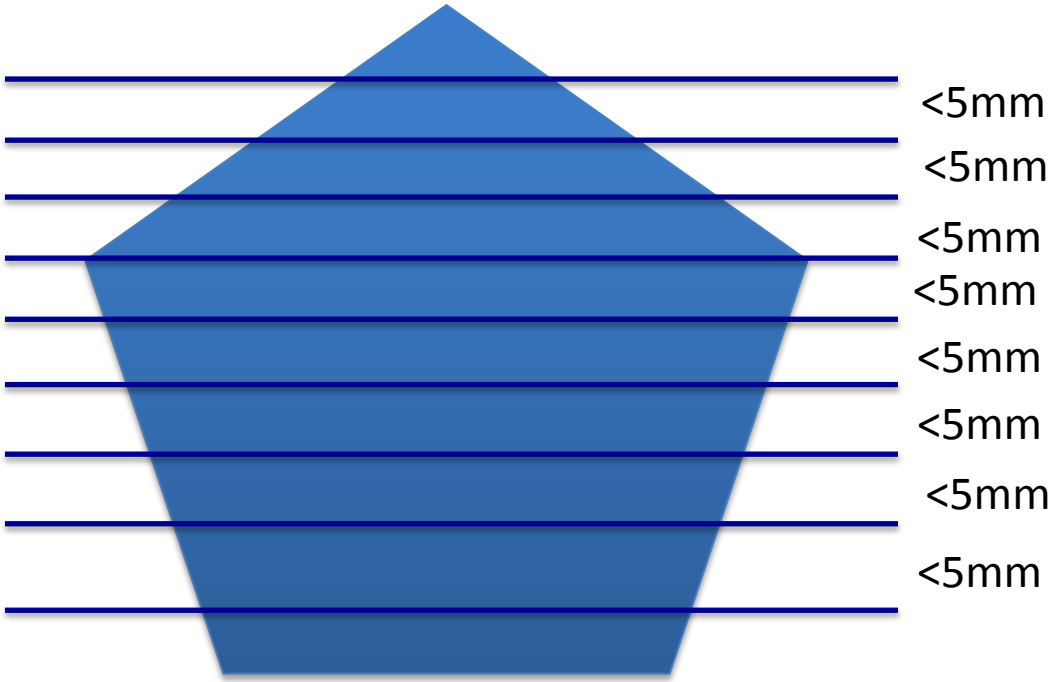
VARIBILI PRE-ANALITICHE CHE INFLUENZANO I DATI ISTOPATOLOGICI E MOLECOLARI

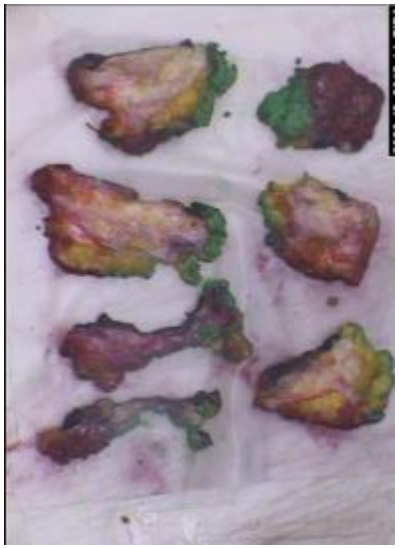
- **VARIABILE «UMANA»**
- **TEMPO**
- **TEMPERATURA**
- **FISSAZIONE**

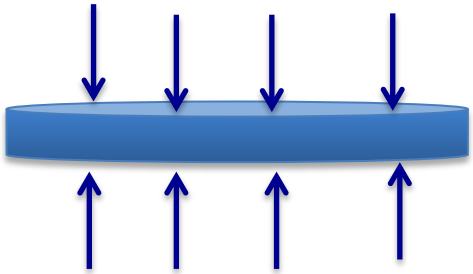
Immersion in formalin of a large specimen is not fixation

v/v 2:1

2 liters of formalin







24-48 h

Formalin penetration and fixation should be completed

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

CONSERVAZIONE E ARCHIVIAZIONE DEL CAMPIONE IN ANATOMIA PATOLOGICA

1. Materiale non processato, cosiddetta “riserva non campionata”

2. Materiale processato ai fini della campionatura

TERMINI TEMPORALI: 10 anni (OBBLIGO)

DECORRENZA: validazione del referto

BLOCCHETTI

MODALITÀ: Garanzia della tracciablità

LUOGO: ambienti a temperatura (<27°C) e umidità controllata (>30% e <70%), con sistemi di controllo per l'infestazione da parassiti.

VETRINI

MODALITÀ: garanzia della tracciabilità, si auspica la possibilità di inserire sistemi di conservazione digitale

LUOGO: ambienti e/o sistemi adatti a garantirne la sicurezza, la tracciabilità e la conservazione idonea per eventuale revisione.

CONCLUSIONI

Dal prelievo all'analisi molecolare: certezze e criticità

CERTEZZA: LA GESTIONE DEL TESSUTO DIVENTA CRUCIALE NELL'ANATOMIA PATOLOGICA DEL PRESENTE E DEL FUTURO

CRITICITA': LE FASI PIU' CRITICHE NELLA CORRETTA GESTIONE DEL TESSUTO RISIEDONO PRINCIPALMENTE NELLA PRE-ANALITICA, PRIMA DELL'OSSERVAZIONE DIAGNOSTICA AL MICROSCOPIO

LA SCRUPOLOSA OSSERVANZA DEL DOCUMENTO DEL MINISTERO DELLA SALUTE NEL TENTATIVO DI **STANDARDIZZARE** LE CRITICITA' PUO' RAPPRESENTARE UNA **SOLUZIONE** UTILE SIA AL PAZIENTE CHE ALLA RICERCA SCIENTIFICA