

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



Pre-analytical issues in pathology

3rd TASTE Workshop Proceedings Deliverable 7.8

**Porto, Portugal, 19th October 2013
IPATIMUP**

Preface

The following presentation contains the contributions presented at the Third TASTE workshop, “Pre-analytical issues in pathology”, held on the 19th of October 2013 in Porto, Portugal. The workshop has been developed 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 system</i> Dr. Emanuela Ovcin, COREP, Turin, Italy	» 5
<i>Experience in Vacuum preparation for routine specimens</i> Prof. Gianni Bussolati, COREP, University of Turin, Turin, Italy	» 11
<i>What we can do with vacuum fixation material?</i> Isabella Castellano, University of Turin, Turin, Italy	» 48
<i>Pre-analytical issues for molecular studies</i> MD, PhD Maria Comanescu, Victor Babes Institute of Research and Diagnosis, Bucharest, Romania	» 76
<i>Pre-analytical issues and Liquid-Based Cytology</i> Francesco Feoli, Institut Jules Bordet, Bruxelles, Belgium	» 95
<i>Pre-analytical issues and Tissue Microarrays</i> Manuel Salto Tellez, Centre for Cancer Research and Cell Biology, Queen's University, Belfast, North Ireland	» 107
<i>Tissue heterogeneity as a pre-analytical source of variability</i> Ana Justino, IPATIMUP, Porto, Portugal	» 148

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. Standardized optimal technical quality is certainly important on every day practice of every pathology department. 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 third TASTE workshop will focus on the importance of pre-analytical issues in the quality of the histological and cytological material as well as in the application of ancillary techniques in these materials, illustrating the benefits of standardization.



Lifelong Learning Programme



The TASTE system

Emanuela Ovcin

COREP

Third TASTE Workshop, Porto, 19 Oct 2013

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



The TASTE Background

- Histological and cytological preparations are not standardized and their **quality** level is variable.
- This can affect diagnoses



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

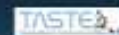
The TASTE project goals



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**
- identifying **targets** and their **needs**
- creating the **TASTE Virtual Community**



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

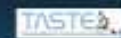
The TASTE system



The TASTE system is based on the open source software **Moodle** linked to a **virtual microscope system**, accessible through Internet, based on **Olympus dotSlide** system with NIS database;

Images are acquired at around **40.000/160.000 dpi** (a normal photo is acquired at 600 dpi).

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.

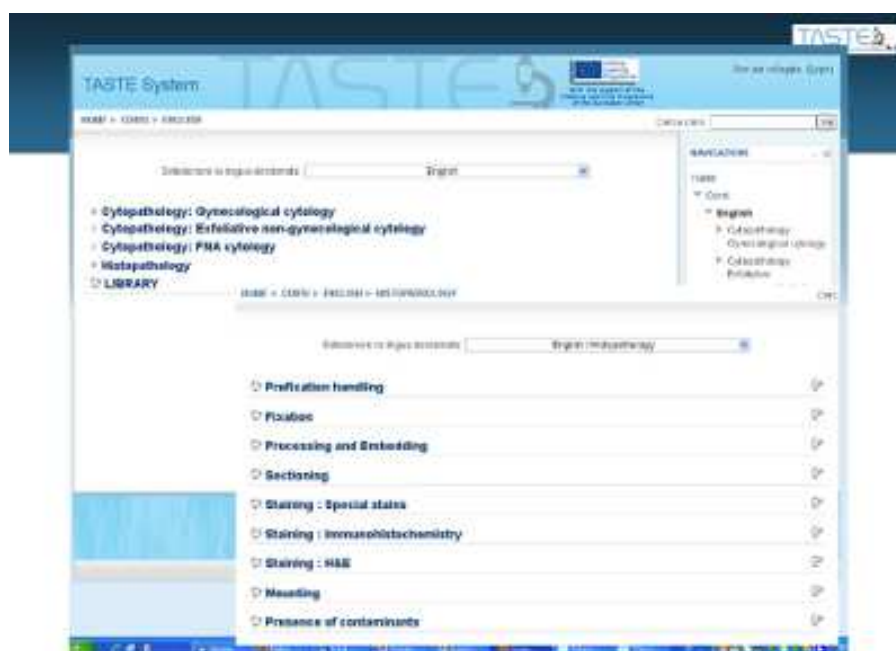


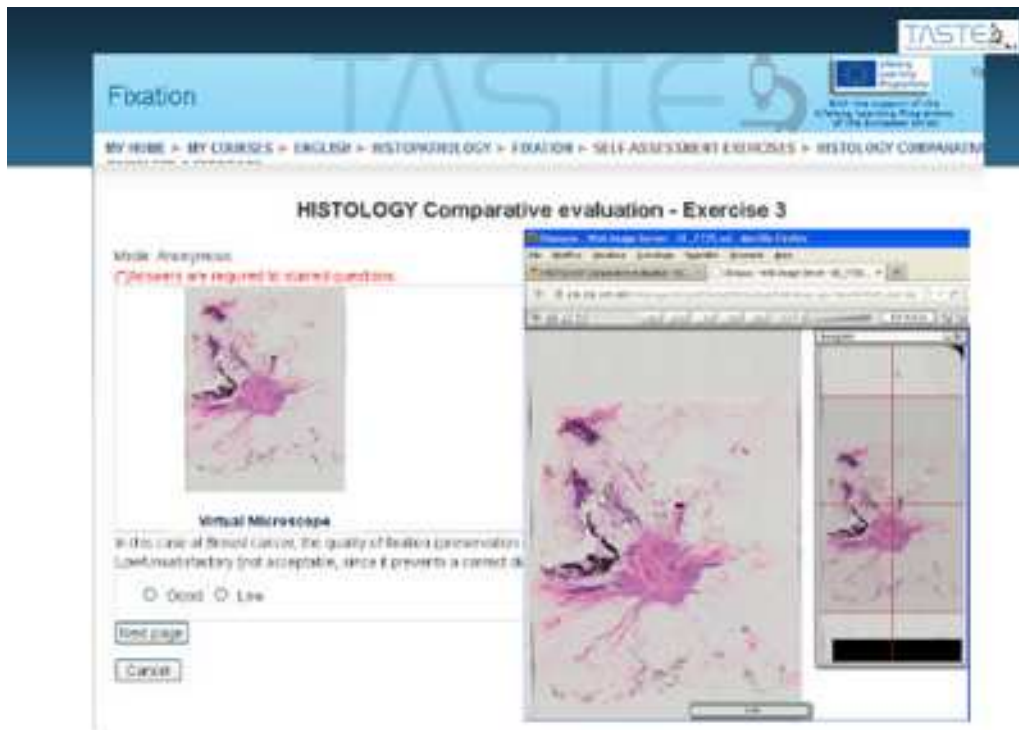
Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency





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 January/February



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

The TASTE Virtual Community



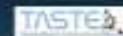
It is expected that the present approach will fuel a Web-based **TASTE Virtual community**.

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

EACEA

Education, Audiovisual & Culture
Executive Agency

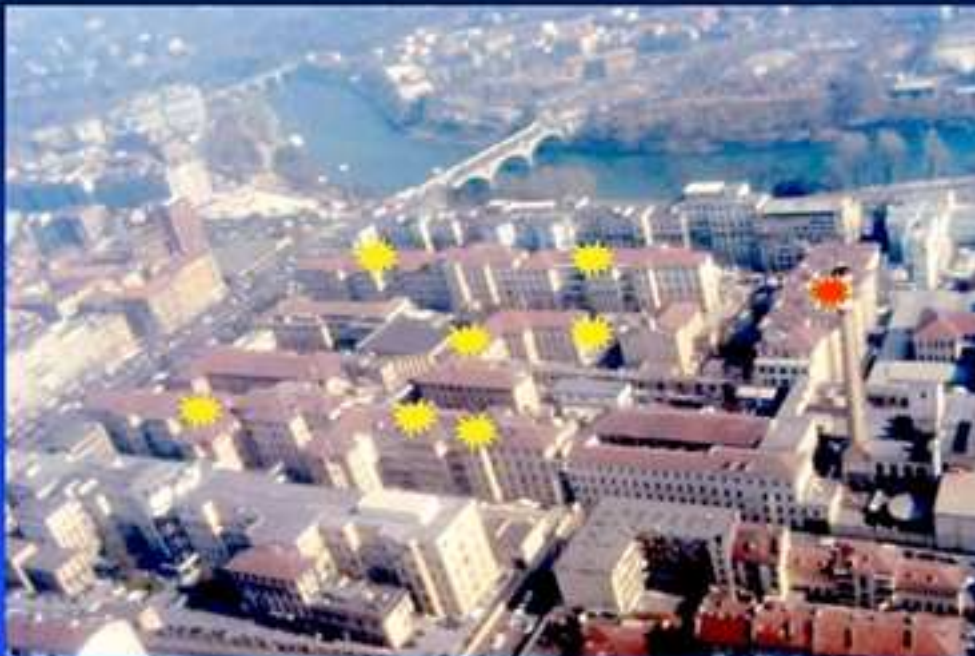


Experience in Vacuum preparation for routine specimens

Gianni Bussolati

University of Turin

Porto, Portugal, 19th October 2013





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



Molinette Hospital
year 2005.....
once a day at 2pm

Jan. 2006:

**Surgeons start to operate on
Friday afternoon.**

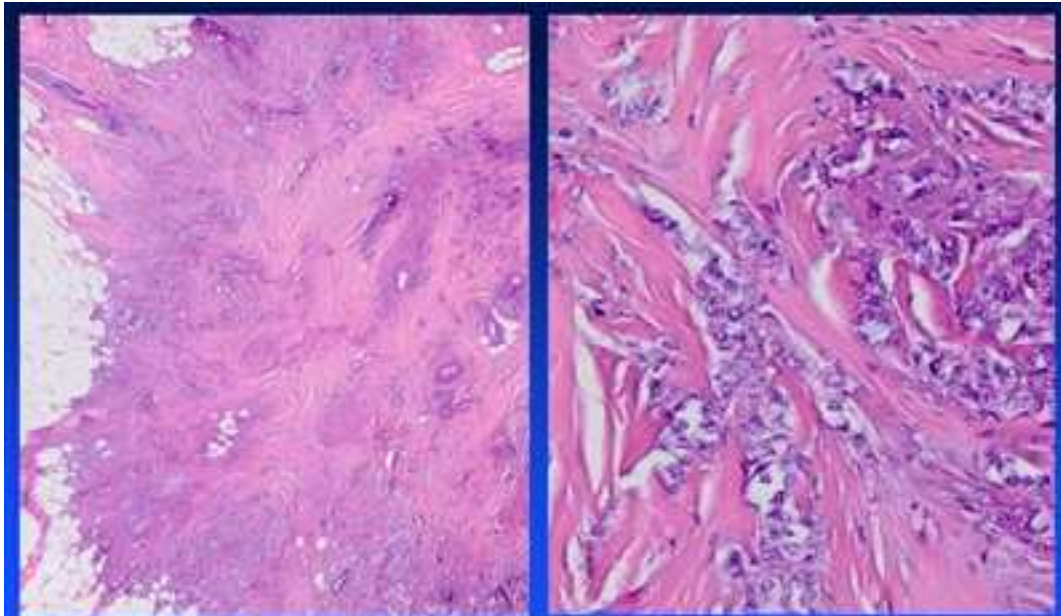


Education and Culture DG

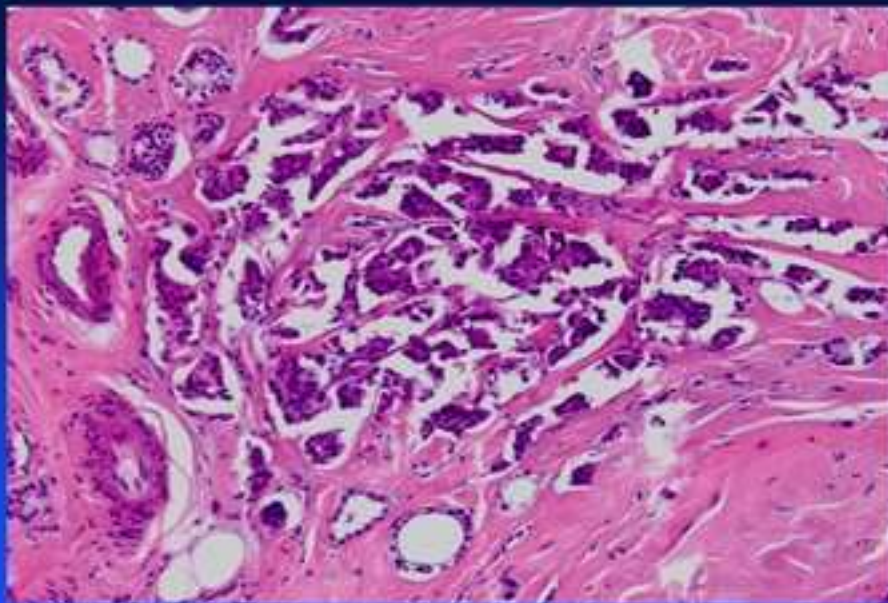
Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Breast Cancer. Operated on Friday, Immersed in Formalin, Arrived on Monday.
Jan. 2006



Breast Cancer. Operated on Friday, Immersed in Formalin, Arrived on Monday.
Jan. 2006

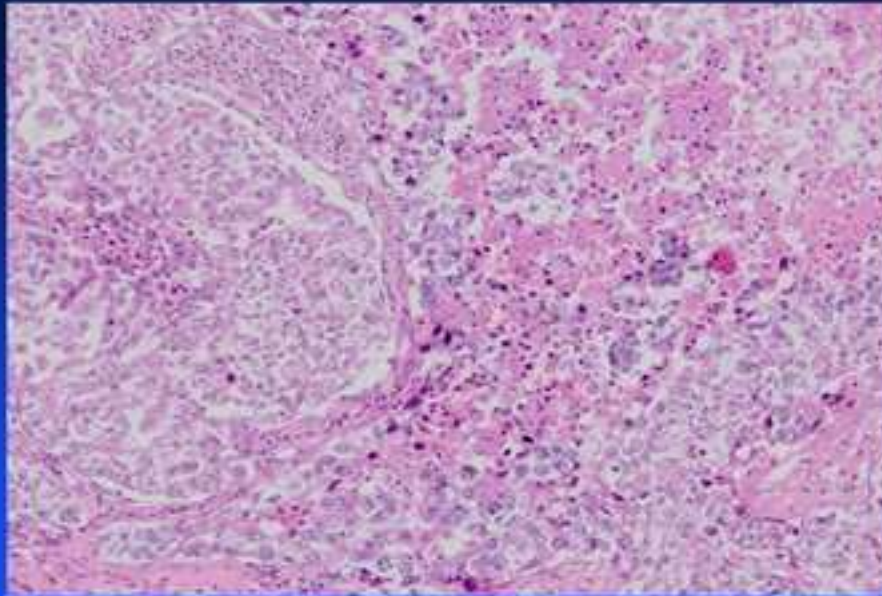


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Breast Cancer Operated on Friday, Immersed in Formalin, Arrived on Monday.
Jan. 2006

- 1) Pre-analytical step (warm /cold Ischemia time)
- 2) Under Vacuum Sealing and Cooling (UVSC)
(a problem and its solution)



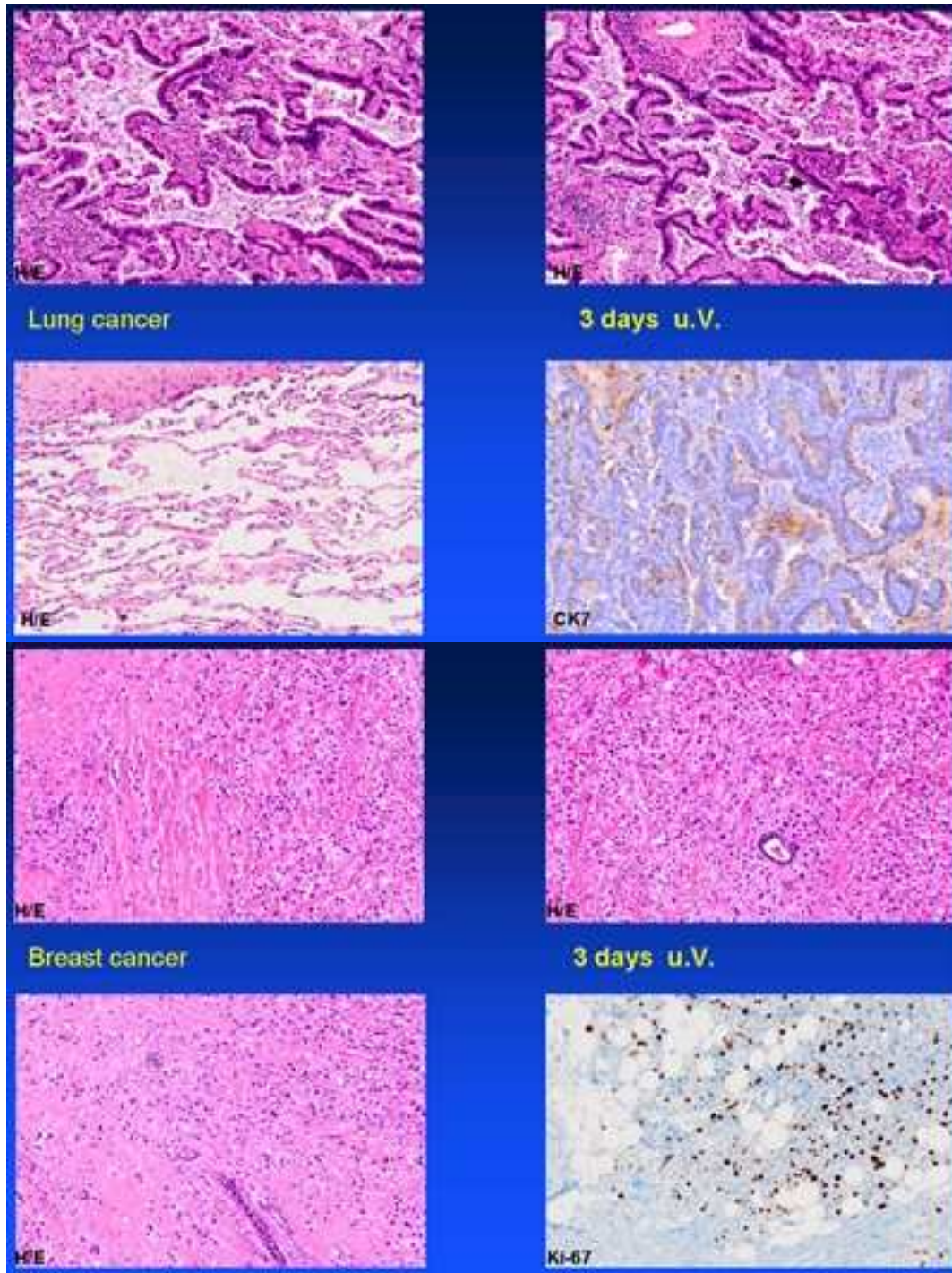
Education and Culture DG

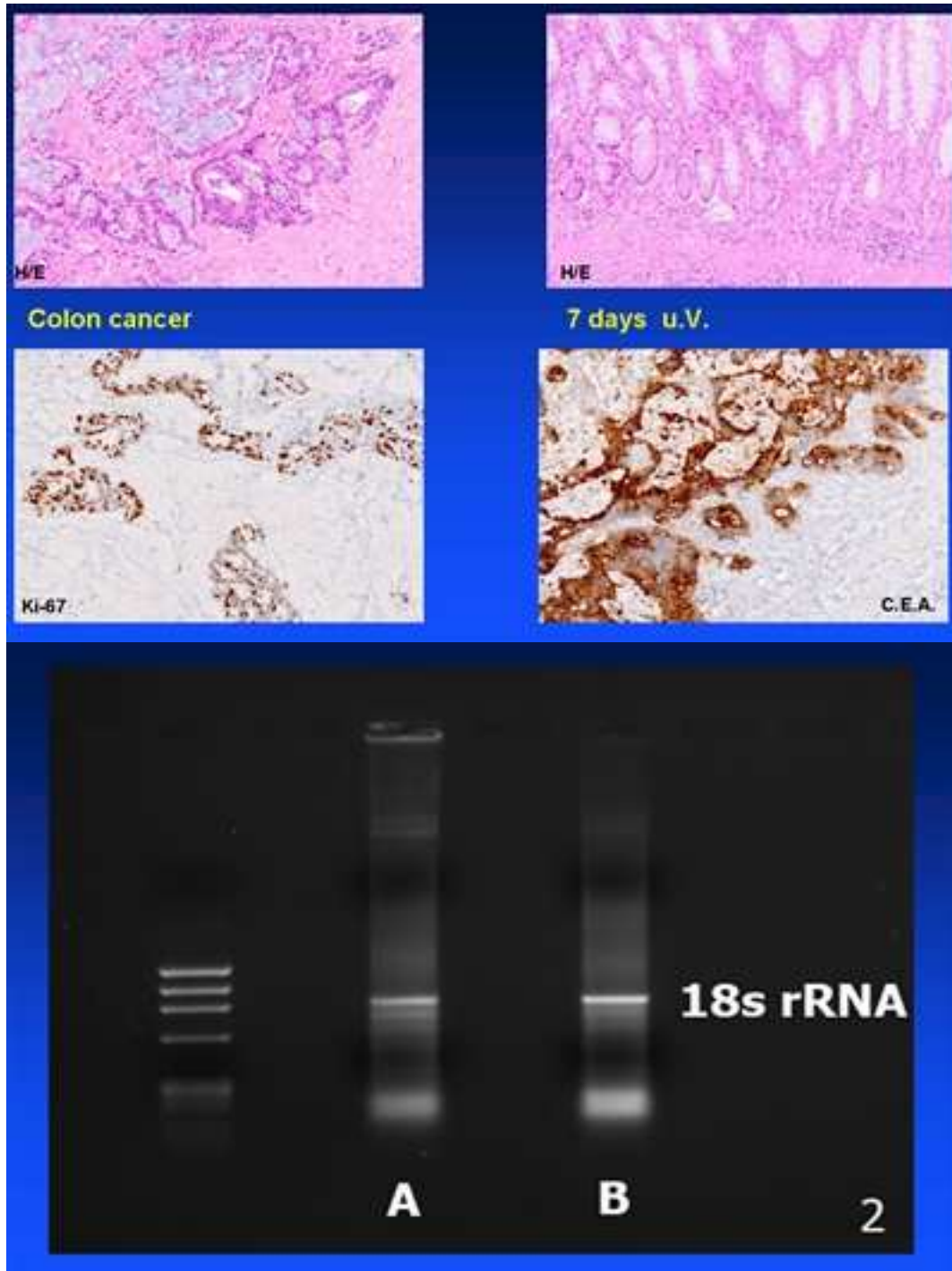
Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency







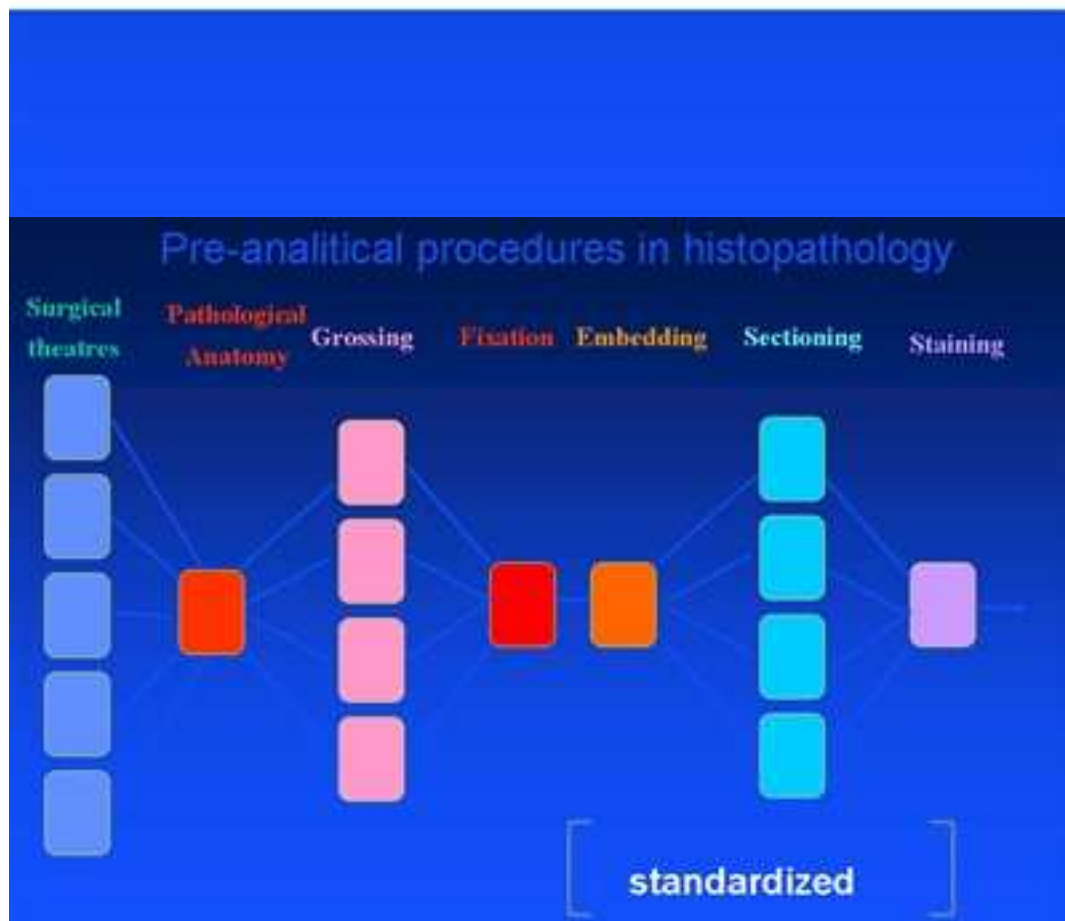


Virchows Arch
DOI 10.1007/s00428-007-0529-x

LETTER TO THE EDITOR

Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin

Gianni Bussolati • Luigi Chiusa • Antonio Cimino •
Giuseppe D'Armento





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Optimal, standardized procedures are crucial if a high standard of test results is to be achieved, which is what each patient deserves.

Groenen et al., Histopathol. 59, 1-7, 2011

•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".



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Basis of histopathology

- Preservation
 - Structure
 - Proteins
 - DNA / RNA

“garbage in / garbage out”

AIMS

Preservation of

- Structure (morphological diagnosis)
- Proteins (Immuno-histochemistry)
- Nucleic Acids (FISH + GEP)



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Pre-Analytical Time Interval (PATI)

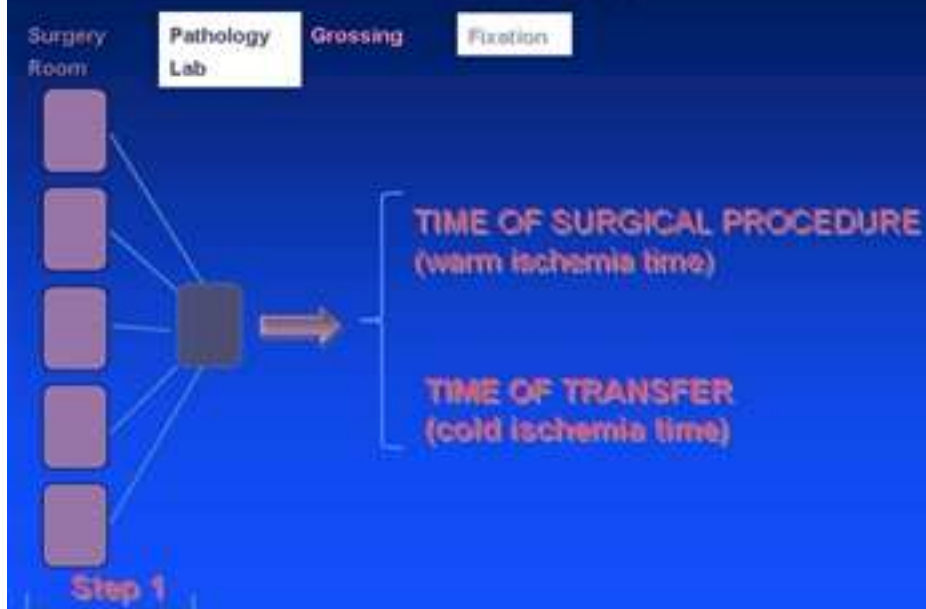
**Interval 1: From the body to the surgical table
(Temperature 37°C or more)**

"warm ischemia time"

Depends on:

- a) Type of operation**
- b) Modality of intervention**
- c) Ability of the surgeon**

PREANALYTICAL PROCEDURES IN SURGICAL PATHOLOGY





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

Interval 2: From the surgical table to the pathology laboratory

"cold ischemia time"

Alternatives :

a) Tissues transferred "fresh" at room temp. (20°C)



How cold is cold ischemia?

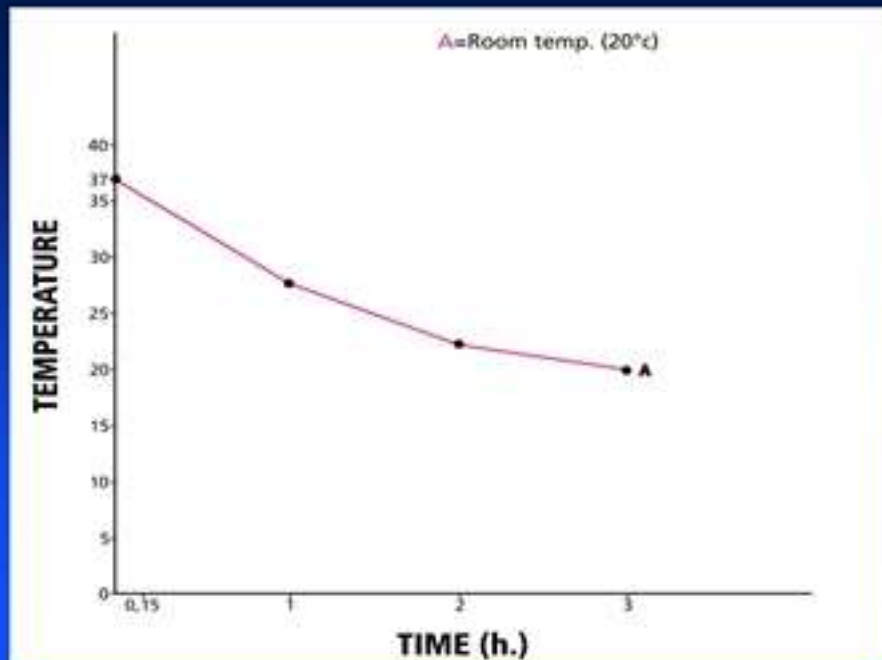


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Interval 2 -From (B) to (C):

Alternative: a) Tissues left fresh

Merits:

- No Fixation

(material available for banking)



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Interval 2 -From (B) to (C):

Alternative: a) Tissues left fresh

Dangers:

- **Effect of delay on:**
 - **Structure**
 - **Proteins (antigens)**
 - **Nucleic acids**

ASCO[®] / CAP Guidelines for Breast Cancer Fixation

- 1) Reduce time of “tissue ischemia”
before grossing - fixation to < 1 h**



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Interval 2: From the surgical table (B) to the pathology lab (C)

Alternatives :

- a) Tissues left fresh
- b) Tissues immersed in formalin

Formalin Fixation:

Advantages

- Cost
- Safe for tissue
- Fast penetration
- Excellent morphology
- ICC

Disadvantages

- Unsafe for operators
- Effect on Nucleic Acid



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Interval 2 -From (B) to (C):

Alternative: b) Tissues immersed in formalin

Temperature: Room Temperature (generally)

- Time interval : from a few minutes up to days.

Formalin: penetration is fast initially (1mm/h), then much slower (1cm/24h).

This is followed by fixation (slow);
subtotal binding plateau at 24 h.

Formalin penetration time





Formalin penetration time

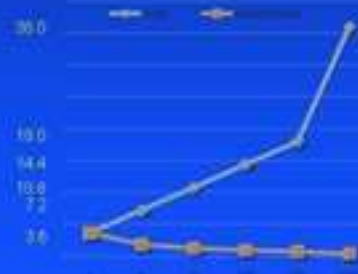


Author: Geoffrey Rods
Institute: Lyce Biosystems, Wetzlar,
Germany 06. March 2012

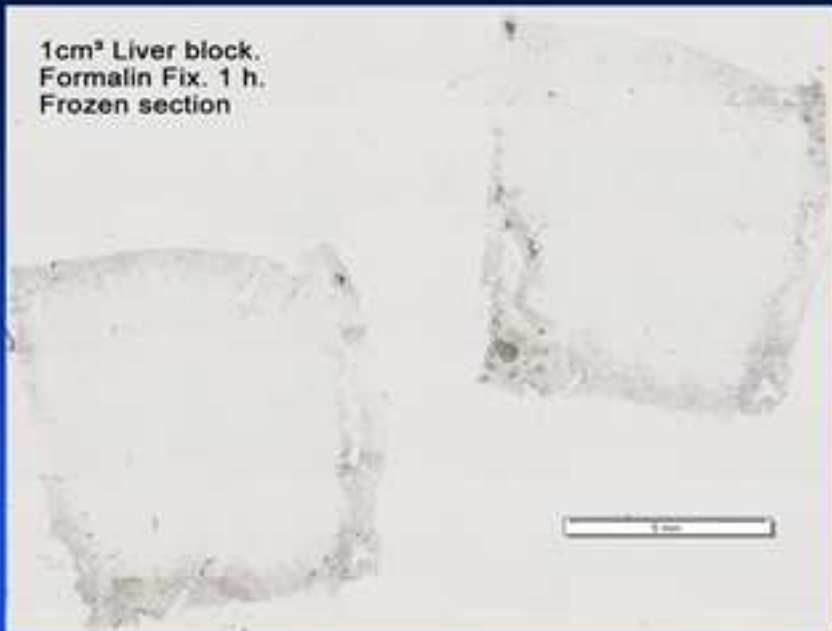
The first layer of cells (20 microns) takes a second. (Bakers $K = 3.6$) depth of penetration, (d) = $K\sqrt{t}$ (K is the coefficient of diffusion), (T is time)

Bryan R. Hewitt
Technical Specialist
Anatomical Pathology
Hamilton Regional Laboratory Medicine Program
Ontario, Canada

hours	mm/hours	mm
1	3.6	3.6
4	1.8	7.2
9	1.2	10.8
16	0.9	14.4
25	0.72	18
100 (4 days)	0.36	36



1cm³ Liver block.
Formalin Fix. 1 h.
Frozen section



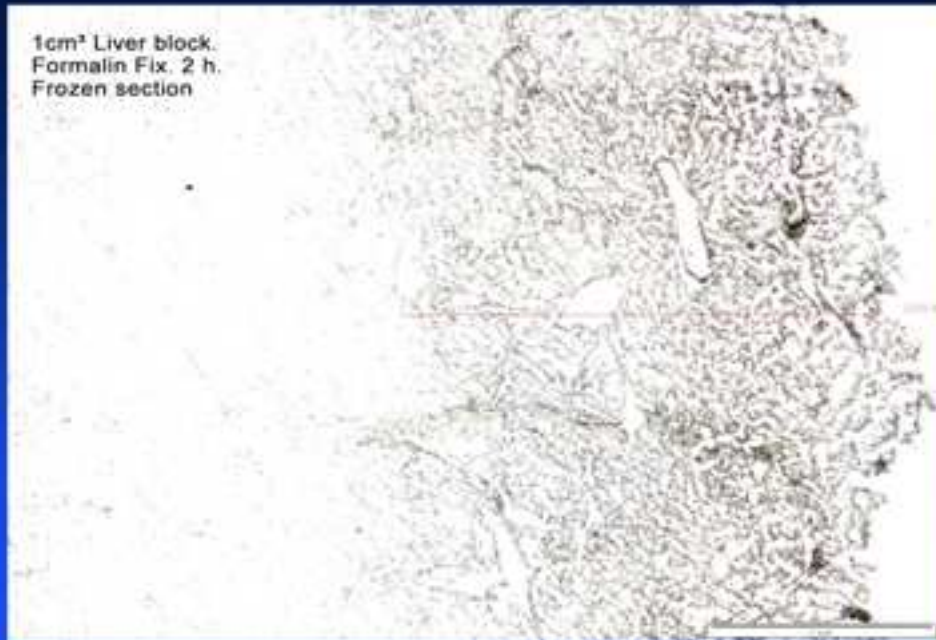


Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



Interval 2 -From (B) to (C):

Alternative: b) Tissues immersed in formalin

Merits:

- In small blocks it rapidly affects:
structure, antigens and nucleic acids
(preservation /de-naturation)



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Tissues immersed in Phosphate – buffered Formalin Small Biopsies <1 cm. = Uniform fixation

Large specimen (> 2 cm)

- **Outside = Fixed**
- **Inside = Autolysis**



000_13_2012_PROF

Liver specimen. Immersed in Formalin. 24 h.

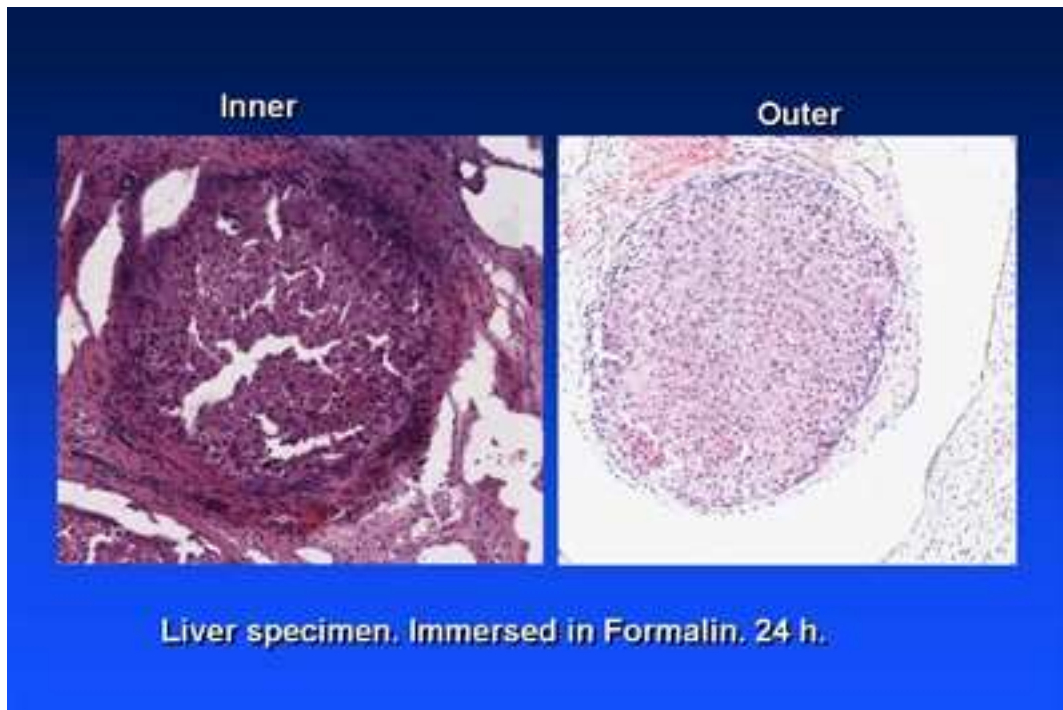


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Interval 2 -From (B) to (C):

Alternative: b) Tissues immersed in formalin

Drawbacks (in large specimens):

- Degradation continues in deep areas
- Tissue banking is hampered
- Formalin containing vessels heavy to carry
- Spilling of formalin may occur
- Fumes dispersed while grossing
- Nurses refuse to handle this “carcinogen” in surgical theatre (and without hoods)
- Tissue forgotten by the surgeon because “already safe in formalin”



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Interval 2: From the surgical table to the pathology lab

Alternatives :

- a) Tissues left fresh
- b) Tissues immersed in formalin
- c) Under-vacuum Sealing and Cooling**

Why vacuum packing and cooling?

Vacuum packing or vacuum packaging is a method of **packaging that removes air (OXYGEN)** from the package prior to sealing. The intent is usually:

- to extend the shelf life of food
- to reduce the volume of the contents and package with flexible package forms.

Depending on the product, the **shelf life** (length of time that a commodity may be stored without becoming unfit for use) or **consumption** of vacuum packaged products can easily exceed 6-times normal bagged or wrapped packages.

Vacuum packing reduces atmospheric oxygen, limiting the growth of **anaerobic bacteria** (e.g., botulism) and **oxidation** (e.g., rancidity, color change).





Result of the IDEA



TissueSAFE
Formalin-free
vacuum sealing
of biospecimens

Milestone





(12) **United States Patent**
Bussolati

(10) Patent No.: **US 8,110,346 B2**
(45) Date of Patent: **Feb. 7, 2012**

(54) **PROCESS FOR PRESERVING TISSUES**

(75) Inventor: **Giovanni Bussolati, Turin (IT)**

(73) Assignee: **Milestone S.r.l. (IT)**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 225 days.

(21) Appl. No.: **12/531,197**

(22) Filed: **Dec. 9, 2008**

(65) **Prior Publication Data**
US 2009/0191533 A1 Jul. 30, 2009

(30) **Foreign Application Priority Data**
Dec. 13, 2007 (EP) 07123113

(51) Int. Cl. **A61N 1/00 (2006.01)**

(52) U.S. Cl. **435/284.1; 27/24.1**

(58) **Field of Classification Search** **435/1.1; 435/284.1; 27/24.1**
See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS

5,695,933 A 8/1997 McWilliams
5,715,585 A * 2/1998 Sandoval
6,739,312 B1 5/2004 Marino

FOREIGN PATENT DOCUMENTS

DE 10155968 A1 5/2003
WO 92/11760 A1 3/1992
WO 00/08219 A1 3/2002

OTHER PUBLICATIONS

Church et al., "Modified atmosphere packaging technology: a review," J. Sci Food Agric. 67:143-152, 1995.*
CVP® System, Fresh Vac® Modified Atmosphere Packaging Machine, A-200, <http://www.cvp-system.com/modula200.htm> printed from the Internet on Sep. 26, 2011.*
CVP® System, Fresh Vac® Modified Atmosphere Packaging Machine, A-200, brochure, <http://www.cvp-system.com/modula200/PDF/A-200.pdf>, printed from the Internet on Sep. 26, 2011.*
The European Search Report issued in connection with EP 07123113.8-1257 on Mar. 3, 2008.
* cited by examiner

Primary Examiner — Resonant Kouson
(74) Attorney, Agent, or Firm — The R.T. Tran Law Group

(57) **ABSTRACT**
A process for preserving tissues is described which comprises transferring the tissues in a container and evacuating the container.





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

VACUUM PACKING and COOLING PROCEDURE:

1. Immediately after excision, specimens are transferred to an adjacent room where the TissueSAFE is installed.
2. The surgical specimen is placed into sterilized specimen bags and sealed under vacuum.
3. Case information are written directly on the printed note field. Starting of vacuum (cold ischemic time) is printed.



Project: To standardize the temperature
of transport and storage

BACKGROUND

Vacuum Sealing and Cooling as Methods to Preserve Surgical Specimens

Kristensen T., Engvad G., Nielsen O., Pless T., Weller S., Blum M.

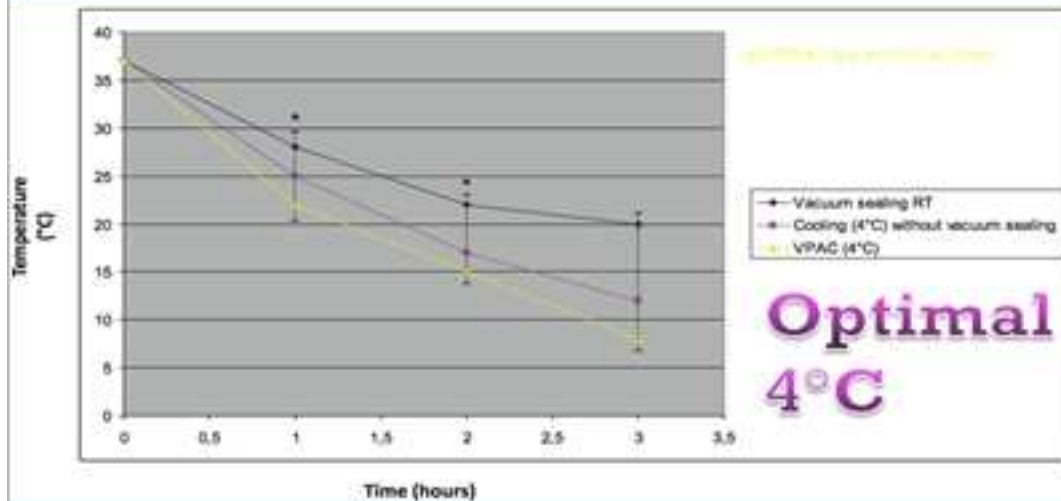
Appl Immunohistochem Mol Morphol. 2011

Storage at 4°C was shown to preserve tissue to a higher degree than storage at room temperature for all included endpoints, independently of whether the tissue was subjected to vacuum sealing or not.



To standardize temperature of vacuum packing and cooling (VPAC)

Annaratone PLoSone 2013



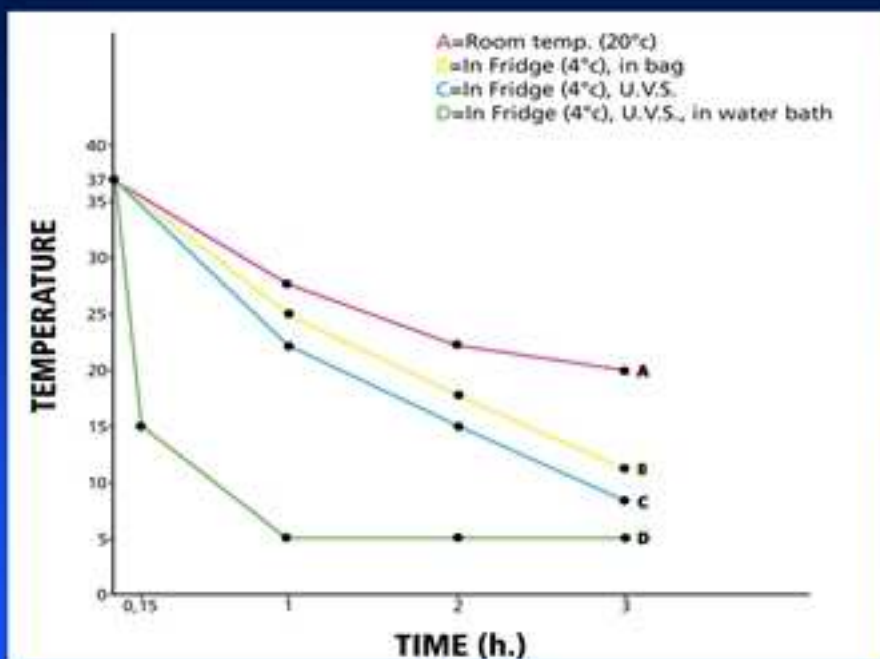


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



SPECIMEN RECEPTION



SPECIMEN STOCKING AT 4°C





Education and Culture DG

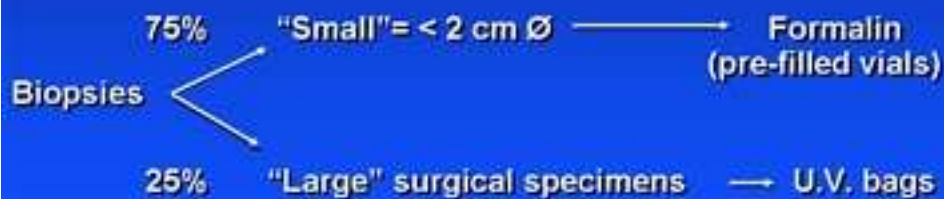
Lifelong Learning Programme



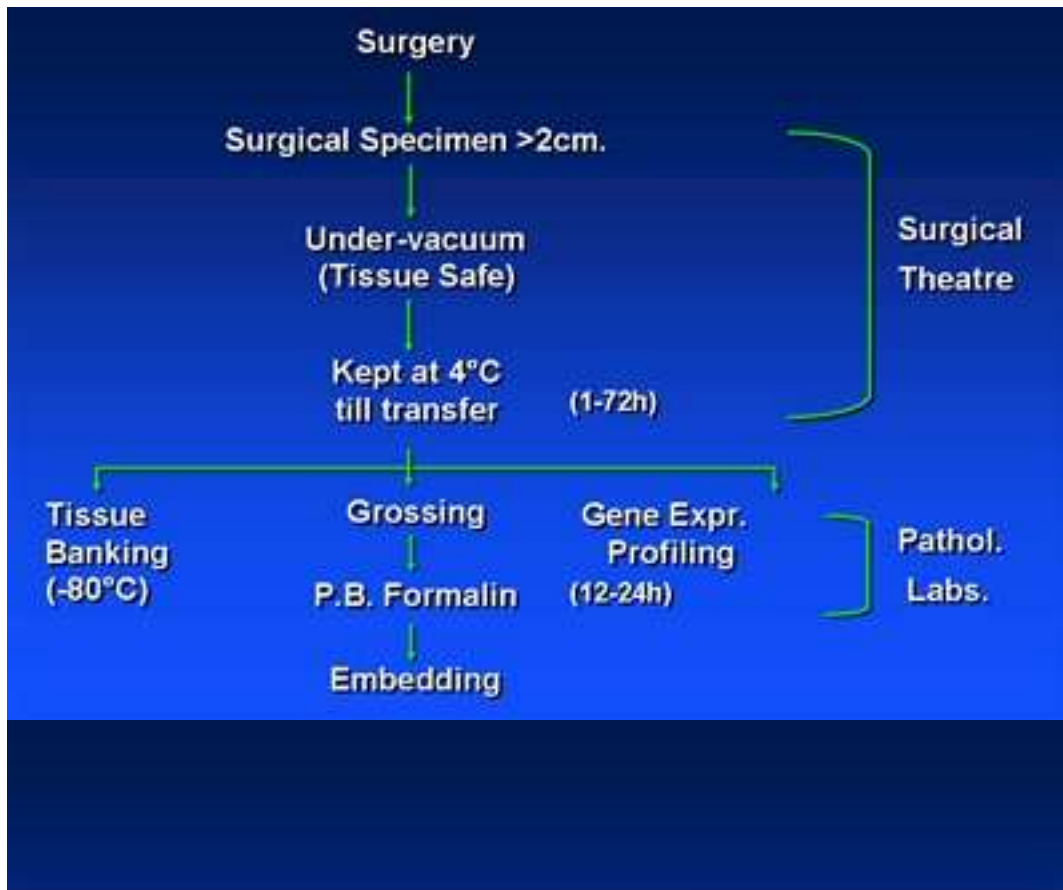
Education, Audiovisual & Culture
Executive Agency

Project: "...Towards a Formalin-free Hospital" "Molinette" Hospital, Turin

- 1162 Beds; >54.000 yearly admissions; > 40.000 histopathological exams. (2008)



Molinette Hospital
year 2010



Science of the Total Environment 406 (2010) 3062–3065



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotene



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

Cinzia Di Novi ^a, Davide Minniti ^b, Silvana Barbaro ^b, Maria Gabriella Zampirolo ^b,
Antonio Cimino ^c, Gianni Bussolati ^{c,*}



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Staff satisfaction

Level of Satisfaction	Freq.		Percent	
	formalin	under-vacuum	formalin	under-vacuum
Low	42	6	39.25	8.57
average	44	17	41.12	24.29
high	21	47	19.63	67.14
Total	107	70	100.00	100.00

C. Di Novi et al. / Science of the Total Environment 408 (2010) 3092–3095

Gross anatomic preservation

	FORMALIN		UNDER-VACUUM	
	Mean	Std. Dev.	Mean	Std. Dev.
# 1= esophagus and stomach				
STRUCTURE	1.108696	0.3146964	2.077778	0.1490712
COLOUR	1.086957	0.2848849	2.956322	0.2061846
CONSISTENCY	1.913043	0.2848849	2.782609	0.4170288
# 2= colon				
STRUCTURE	1.021739	0.147442	2.913043	0.2848849
COLOUR	1.130435	0.3405026	2.955556	0.2084091
CONSISTENCY	1.911111	0.287799	2.652174	0.4815434
# 3= kidney and prostate				
STRUCTURE	1.934783	0.2496374	2.326087	0.4739596
COLOUR	1.173913	0.383223	2.913043	0.2848849
CONSISTENCY	2.021739	0.147442	2.23913	0.431266
# 4= endocrine / thyroid				
STRUCTURE	1.934783	0.2496374	2.326087	0.4739596
COLOUR	1.23913	0.431266	2.934783	0.2496374
CONSISTENCY	2	0.2108185	2.152174	0.3631584
# 5= liver / spleen				
STRUCTURE	1.904762	0.2971018	2.690476	0.4679011
COLOUR	1.190476	0.3974366	3	0
CONSISTENCY	1.97619	0.1543033	2.404762	0.4967958

1= weak
2= satisfactory
3= good

C. Di Novi et al. / Science of the Total Environment 408 (2010) 3092–3095



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Result of Survey among Staff Operators (October 2008 – April 2009)

- Satisfaction:
 - Low for Formalin
 - High for U.V.
- Handling & Gross Anatomy
 - Histopathol. + ICC - U.V. = no drawbacks

Project: ——— F-f Hospital

Alternative c) Tissues preserved under vacuum

Merits:

- No more formalin in surgical theatre (except for small specimens, where pre-filled tubes are employed)
- No spilling
- No fumes
- No drying of tissues
- Colours preserved
- Lack of insulating air around tissues allows fast cooling
- Tissues (bags) light and easy to carry
- Structure (RNA, Antigens) preserved up to days
- Banking (selective) allowed
- Demonstrating of operated tissues is convincing for students and surgeons



Education and Culture DG

Lifelong Learning Programme



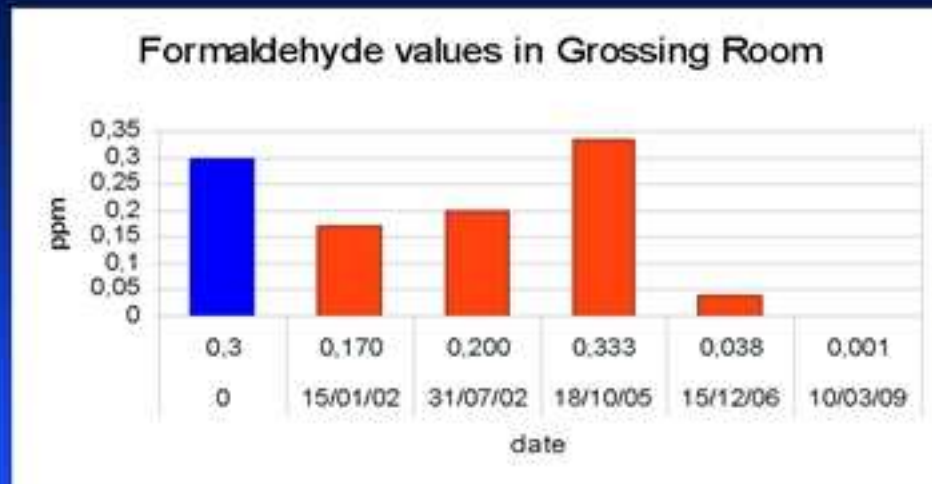
Education, Audiovisual & Culture
Executive Agency

TissueSAFE users' list (Aug 2013)

Molinette Public Hospital, Turin - Italy (7 units)
S. Anna public Hospital, Turin – Italy (2 units)
Ospedale Papa Giovanni XXIII, Bergamo Italy (5 units)
Forlanini San Camillo, Rome -Italy (2 units, in process for 4 more)
Gaslini Public Hospital, Genova – Italy
European Institute of Oncology IEO, Milan – Italy (3 units)
Niguarda Hospital, Milan – Italy (3 units)
Bufalini Hospital, Cesena – Italy (2 units)
S.Maria Hospital, Udine – Italy (2 units)
Public general Hospital, Cremona – Italy (3 units)
IRCCS Verona – Italy (2 units)
Ospedale Pascale, Napoli – Italy (4 units)
Centro Oncologico Fiorentino – Italy
Ospedale Palermo – Italy (5 units)
UMC Univ. Hospital, Nijmegen – Holland (2 units)
Medisch centrum parkstad, Heerlen – Holland
Groeningen – Holland
Public Hospital, Hvidovre – Denmark (3 units)
Public Hospital, Aalborg – Denmark (2 units)

TissueSAFE users' list (Aug 2013)

Hopital Croix Saint Simon, Paris – France
Hopital Europeen Georges Pompidou, Paris – France
Besancon – France (12 units)
Hospital Garcia de Orta, Almada- Portugal (2 unità).
Hospital de São João, EPE (HSJ), Porto – Portugal.
Hospital Universitario Virgen de la Arrixaca, El Palmar (Murcia) – Spain
Hospital Can Ruti, Badalona (Barcelona) – Spain
Hospital Virgen Del Rocío, Sevilla - Spain
Military hospital, Ulm – Germany
Universitätsklinikum Carl-Gustav-Carus, Dresden - Germany
Simmelweis II Institute, Budapest – Hungary
Regional Oncology center, Kursk – Russia
Assaf Harofeh, TelAviv – Israel
Hospital Regional 'Dr. Ernesto Torres Galdames' - Iquique, - Chile
Khon Kean University, Khon Kean City -Thailand



Threshold Limit Value (TLV)

“garbage in / garbage out”

Treasure preserved in



Golden diagnoses out



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Funding Opportunities

- **Project title:**

Standardizing tissue handling in pre-analytical procedures: **technological, environmentally-safe innovation in solid tumour processing.**

FUNDED BY: Ministry of Health in Italy-2012

COST 2013

TITLE: Pre-analytical tissue preparation for clinical diagnostics and molecular pathology. Establishment of a European Network: (PATEN)

WG1: Specimen collection and transfer

WG2: Specimen preservation

WG3: Specimen processing and storage

WG4: Analytes quality standards

WG5: Data management

WG6: Ethical issues



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

AIMS:

- **To improve tissue transfer from surgical rooms to the pathology lab**
- **To improve tissue preservation for:**
 - Histological diagnosis
 - Immunohistochemical tests
 - Molecular tests
 - Research



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

...because of Institution's needs

1

Innovation in Healthcare without Borders – UE Conference-Brussels

Key findings and recommendations

- Healthcare innovations that do not reach patients are a waste of time, expertise, and resources.
- Innovation is not a goal in itself — the goal is to improve healthcare, both in terms of benefits to patients and of economic sustainability, including industrial competitiveness.
- Funding which focuses primarily on pushing technology beyond the state-of-the-art misses the point that patients do not care about what is scientifically sexy, they care about what will provide them benefit in terms of life expectancy and quality of life.

....because of patient's needs

2

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)



Education and Culture DG

Lifelong Learning Programme

EACEA

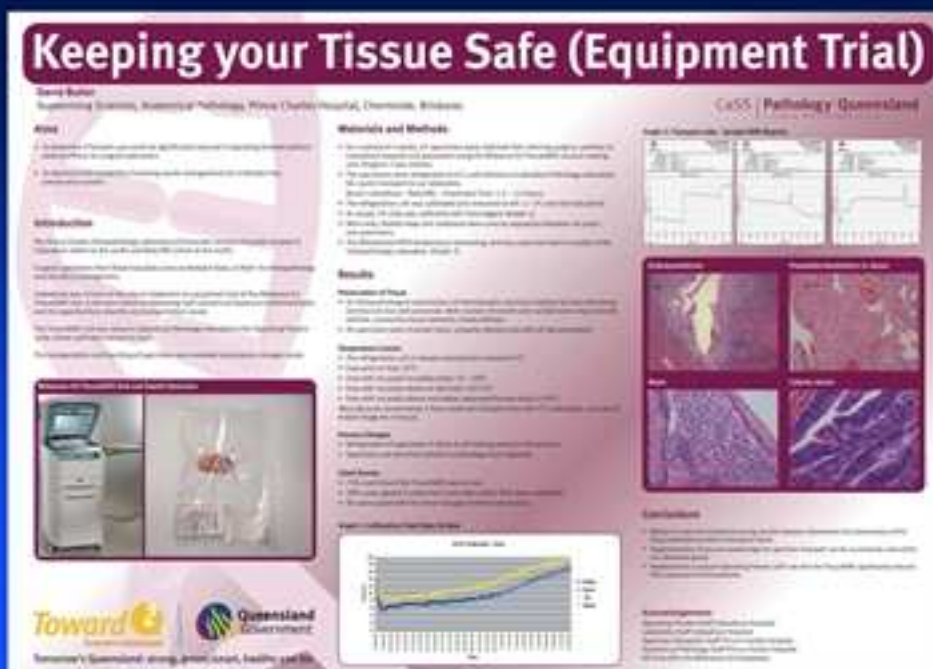
Education, Audiovisual & Culture
Executive Agency

.....because of researcher's needs

3

•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".





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

WHAT TO DO WITH TISSUES PRESERVED UNDER-VACUUM

Isabella Castellano

Department of Medical Sciences

University of Turin, ITALY

Ospedale Città della Salute e della Scienza-

Presidio Molinette

isabella.castellano@unito.it

Surgical Theater: Formalin

Get rid of Formalin from Surgical Theater!



Tissue SAFE





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Pathology Lab: Formalin

But...for the Pathology Lab??



Pathology Lab: current situation

Jar filling procedure is manually performed.

High variability in the specimen/Formalin ratio (no standardization).

High volumes of Formalin for specimens handling (14000 L/Year).

Safety issues related to prolonged Formalin exposure.

Archiving and disposal of biospecimens needs to be improved.





Fomaldehyde: Occupational exposure limits

OSHA*, ACGIH** :

- Short-Term Exposure Limit (STEL, 15min) of **2 ppm**.
- Time-Weighted Average (TWA, 8hr) of **0.75 ppm**.
- Ceiling concentration (Peak) of **0.3 ppm**.



* Occupational Safety & Health Administration (OSHA)

** American Conference of Governmental Industrial Hygienists (ACGIH)

Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production

50 workers from 10 Pathology and Anatomy laboratories.

Ceiling concentrations were higher than reference value (0.3 ppm) in both.

The frequency of Micronuclei in peripheral blood lymphocytes and in nasal mucosa cells was significantly higher than in the control group.

Preventive and protective measures must be applied in order to reduce occupational exposure to this chemical agent to prevent adverse effects on workers health.





Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production

Table 3 FA Ceiling values (ppm) according to places of work, tasks and exposed workers

Places of work	Tasks	Ceiling Values (ppm)	Exposed Workers
Factory Resins production	Sample collect (Reactor)	1.01	Reactor operators
Factory Impregnation	Machine operation	1.04	Impregnation machine operators
Factory Quality Laboratory	Quality control	0.52	Quality Technicians
Pathology and anatomy laboratories	Macroscopic examination	5.02	Pathologist
Pathology and anatomy laboratories	Disposal of specimen and used solutions	0.18	Technicians and Assistants
Pathology and anatomy laboratories	air filling	2.53	Assessors
Pathology and anatomy laboratories	Specimen wash	1.28	Technicians
Pathology and anatomy laboratories	Boiler	1.11	Technicians

Pathologists were the professional group exposed to the highest ceiling concentration values.

Several studies report a carcinogenic effect in humans after chronic exposure to FA, in particular for nasopharyngeal cancer...

Armstrong RW, Imrey PB, Lye MS, Armstrong MJ, Yu MC, Sani S:

Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. *Int J Epidemiol* 2000;29:991-998.

Vaughan TL, Stewart PA, Teschke K, Lynch CF, Swanson GM, Lyon JL, Berwick M:

Occupational exposure to formaldehyde and wood dust and nasopharyngeal carcinoma. *Occup Environ Med* 2000; 57:376-384.

Hildesheim A, Dosemeci M, Chan CC, Chen CJ, Cheng YI, Hsu MM, Chen BJ, Mittl BF:

Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev* 2001;10:1145-1153.

Coggon D, Harris EC, Poole J, Palmer KT:

Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. *J Natl Cancer Inst* 2003; 95:1608-1615.

Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A:

Mortality from solid cancers among workers in formaldehyde industries. *Am J Epidemiol* 2004; 159:1117-1130.



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

How much Formalin is enough to fix tissues?

The volume of Formalin needed to fix a specimen is not generally agreed.

Histotechnology text books and journals articles recommend from **1:0.5 to 1:200** depending on the tissue involved and the proposed study.

Using less Formalin than at the present levels of **1:10 or more** will improve the safety of the AP lab while still permitting the use of this invaluable fixative.



Sperimental assessment

Evaluation of the minimum Formalin amount to
fix and preserve tissues in *under vacuum bags*.

PART 1: UNDER VACUUM FIXATION

- All tests have been carried out on surgical specimens transported to the laboratory under vacuum. Small tissues initially, surgical specimens after.

PART 2: UNDER VACUUM PRESERVATION OF RESERVES

- After grossing, the specimens have been vacuum sealed again in the same fixative. After 2 months, the specimens have been processed to evaluate the preservation of structural characteristics.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

1- Under vacuum fixation

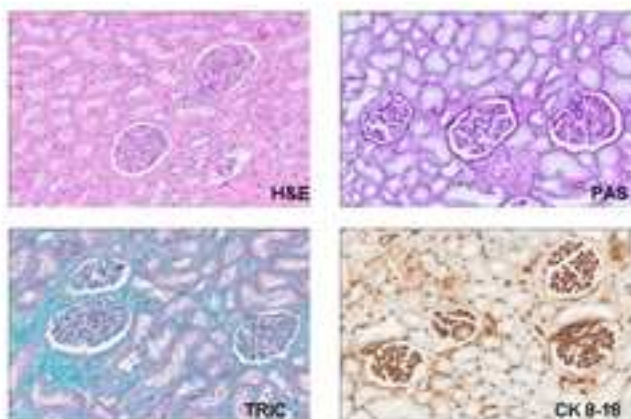
Specimen/Formalin ratio

under vacuum



1:2

1- Under vacuum fixation



Fixation was complete in all cases
no morphological alterations have been found.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

2 - Preservation of anatomical reserves

Surgical specimens need to be stored for up to 2-3 months from the time of diagnosis (for any revisions required by clinician and for medical-legal disputes)



ARCHIVING

2 - Preservation of anatomical reserves





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

Archiving: related drawbacks

Limited space, high volumes required.

Need of aspirated cabinets with
fumes extraction systems.

Need of dedicated personnel for room
cleaning and specimens disposal.

Formalin vapors in the environment
(searching for cases to be reviewed).



Seal SAFE



Automatic filling and vacuum sealing system





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

Seal SAFE

Specimen positioning



Seal SAFE

Automatic specimen weight detection





Seal SAFE

Dispensing the right amount of fixative
into the bag



Seal SAFE

Vacuum sealing of bag





Lifelong Learning Programme



Seal SAFE

All phases with closed and vented instrument



Seal SAFE bag

Practical tissue handling, sealing and archiving





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Benefits



Standardization and Quality

Always the right amount of Formalin according to the specimen weight.
Optimized fixation and histological results.

Less Formalin

Reduction of volumes in AP thanks to a decreased specimen/Formalin ratio.



Safety

Full automation prevents exposure of technical staff to Formalin.

Benefits



Improved archiving

Storage in UVS bags dramatically reduces volume occupied.
Archiving even without Formalin!



Improved disposal

Lower disposal cost thanks to a decreased volume of bio-hazard material in UVS bags.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



GENE EXPRESSION AND
IMMUNOHISTOCHEMISTRY

CELL CULTURES
STEM CELL ISOLATION

TISSUE BANKING

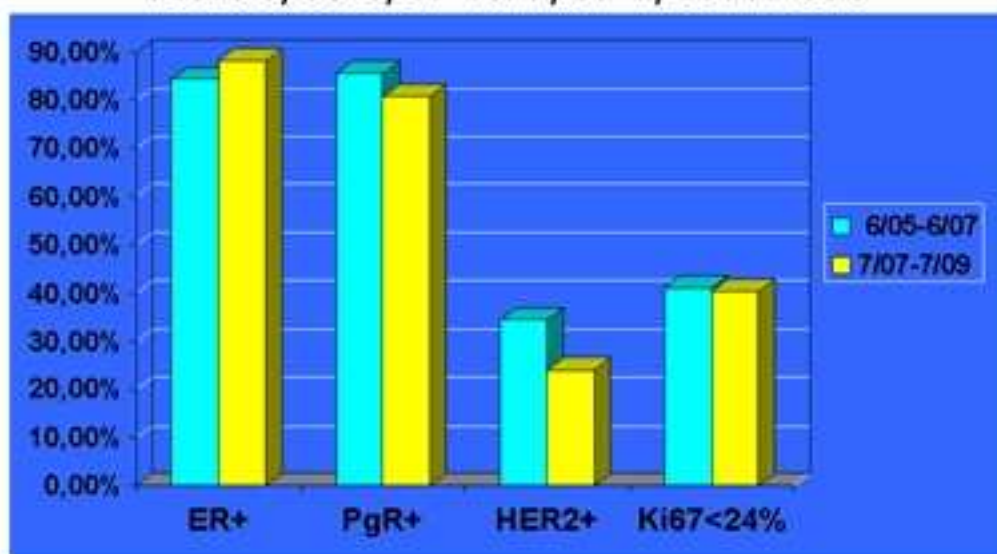


GENE EXPRESSION AND
IMMUNOHISTOCHEMISTRY



ICC evaluation of therapeutic/prognostic parameters in a continuous series of breast cancers

Years 6/05-6/07 vs. 7/07-7/09. N=375



... Using expression levels of 3 epitopes, we can begin to assess the likelihood of delayed time to fixation or decreased tissue quality.

A Tissue Quality Index – an Intrinsic Control for Measurement of Effects of Preanalytical Variables on FFPE Tissue

2013, sent for publication

Veronique M. Neumeister, Fabio Parisi, Allison M. England, Summar Siddiqui, Valsamo Anagnostou, Elizabeth Zarrella, Maria Vassilakopoulou, Yalai Bai, Sasha Saylor, Anna Sapino, Yuval Kluger, David G. Hicks, Gianni Bussolati, Stephanie Kwei and David L. Rimm



Italian Breast Cancer Series (IBC): Tissues from 100 breast cancer cases were collected and time until formalin fixation of these tissues was recorded, ranging from 1 to 72 hours.
...tissues were vacuum sealed and stored at 4 degree Celsius (UVSC) right after surgical removal until gross dissection and immersion of the tissue into formalin.

**Results: The TQI performance on the IBC series
..result in significantly less epitope degradation.**

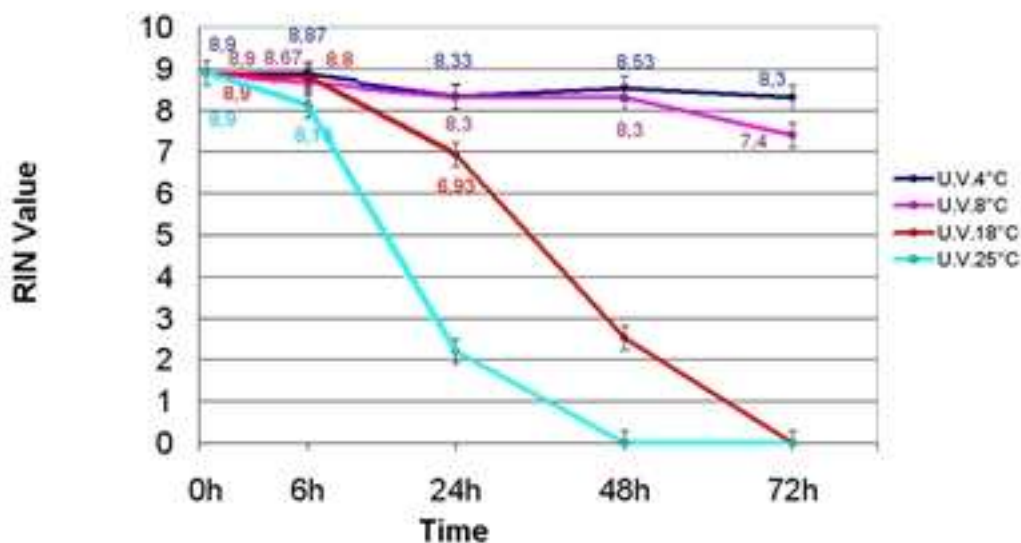
**better preservation of the tissue through
vacuum sealing and storage at 4° C**

**A Tissue Quality Index – an Intrinsic Control for Measurement of
Effects of Preanalytical Variables on FFPE Tissue**

Neumeister et al. 2013, sent for publication

Validation test

RIN Rat Liver U.V.





Breast cancers processed by USV and cooling (4°C)

histo ID	gap	RIN	histo ID	gap	RIN	histo ID	gap	RIN	histo ID	gap	RIN
1285/09/3	1h	9	6054/09/3	6h	8,6	3400/09/3	24h	8,7	8687/09/3	7h	8,6
1385/09/3	60h	7,9	6056/09/3	24h	7,3	3673/09/3	1h	9,6	9000/09/3	24h	7,4
1394/09/3	60h	7,9	6058/09/3	24h	7,9	3657/09/3	72h	7,5	9112/09/3	24h	7,8
1599/09/3	60h	7,4	6226/09/3	2h	6,9	3701/09/3	24h	7	9182/09/3	4h	8,1
1271/09/3	1h	7,8	6263/09/3	5h	7,8	3764/09/3	48h	6,6	9183/09/3	7h	6,9
1498/09/3	5h	7,5	6368/09/3	72h	7,6	3889/09/3	72h	7,8	9248/09/3	48h	8
1469/09/3	5h	7,9	6369/09/3	72h	8	3888/09/3	72h	8,2	9324/09/3	24h	8,7
1422/09/3	2h	8,7	6422/09/3	24h	7,1	4014/09/3	2h	7,6	9452/09/3	5h	7,9
1267/09/3	5h	8,2	6520/09/3	24h	7,7	4073/09/3	6h	9,1	9461/09/3	7h	7,5
1698/09/3	24h	8,7	6552/09/3	24h	7,1	4075/09/3	5h	7,9	9585/09/3	24h	8,6
1783/09/3	60h	7,9	6753/09/3	24h	7,8	4122/09/3	20h	7,3	9687/09/3	4h	7,1
1795/09/3	60h	7,6	6849/09/3	72h	7,8	4221/09/3	70h	7,4	1087/10/3	5h	7,7
1844/09/3	1h	8,7	6907/09/3	24h	8,7	4211/09/3	70h	6,2	1433/10/3	5h	8
1886/09/3	1h	8,5	7004/09/3	6h	8,3	4275/09/3	7h	9,4	305/10/3	2h	7,2
1904/09/3	24h	7,3	723/09/Domen	2h	8,6	4298/09/3	24h	6,6	435/10/3	5h	8,2
1992/09/3	5h	7,3	7129/09/3	6h	8,1	4471/09/3	48h	8,6	506/10/3	6h	8,6
1995/09/3	72h	8,1	7249/09/3	2h	9,1	4553/09/3	30h	8	651/10/3	5h	8,5
2032/09/3	7h	7,5	7250/09/3	4h	9,6	4594/09/3	72h	6,2	650/10/3	3h	6,8
2163/09/3	60h	8,1	7362/09/3	6h	9,4	4617/09/3	2h	8,9	649/10/3	23h	6,8
2241/09/3	1h	8,9	7405/09/3	6h	7,9	4668/09/3	24h	8,6	1022/10/3	72h	8,6
2238/09/3	6h	8,4	7489/09/3	5h	9,1	4831/09/3	5h	8,3	1267/10/3	7h	7,8
2237/09/3	5h	8	7514/09/3	24h	8	4937/09/3	72h	8	1316/10/3	5h	7,7
2574/09/3	6h	7,9	7613/09/3	6h	7,3	5038/09/3	24h	8,2	1453/10/3	24h	6,5
2621/09/3	24h	7,6	7781/09/3	8h	7,9	5118/09/3	72h	6,2	1677/10/3	6h	9
2622/09/3	24h	6,8	7782/09/3	6h	7,7	5357/09/3	24h	7,3	1775/10/3	7h	9,2
2712/09/3	72h	7,4	7805/09/3	5h	7	5525/09/3	5h	7,8	1944/10/3	7h	8
2865/09/3	20h	7,8	7886/09/3	5h	7,9	5598/09/3	1h	7,9	1945/10/3	4h	7,4
2743/09/3	72h	6,4	7904/09/3	24h	8,6	5661/09/3	7h	7,1	2033/10/3	5h	9,1
2820/09/3	24h	7,2	8153/09/3	7h	7	5764/09/3	5h	6,2	2036/10/3	24h	7,4
2817/09/3	24h	7,9	8215/09/3	72h	9	5822/09/3	5h	7,4	2096/10/3	24h	9,5
3216/09/03	1h	7	8413/09/3	72h	6,5				2082/10/3	5h	6,6
3365/09/3	7h	8,1	8542/09/3	5h	7,2						
3368/09/03	7h	7,7	8623/09/3	5h	7,6						

129 consecutive cases of breast cancer, stored in UVS at 4°C for a time between 1 and 72 hours (means 23h), before grossing.

A specimen (punch biopsy) was taken, immersed in RNA later® and sent for Gene Expression Analysis (GEA).

Evaluation of RNA values proved that in all cases the material was fit for GEA analysis (RIN value mean 7.9)



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

**GENE EXPRESSION PROFILING IN UNDER-VACUUM STORED
BREAST CARCINOMAS: A PILOT STUDY**

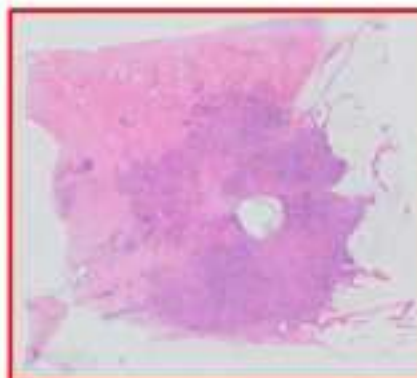
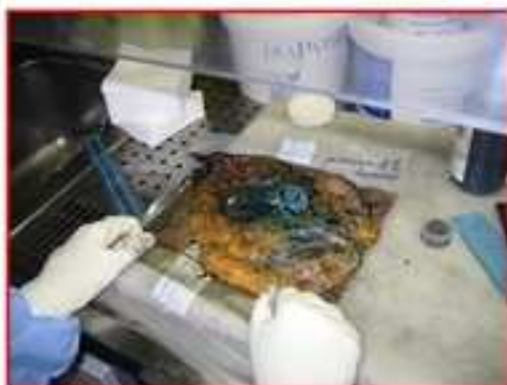
Sapino Anna, D'Armento Giuseppe, Molinaro Luca, Macri' Luigia, Cavicchini
Ada, Bussolati Gianni

**MAMMAPRINT IS A FDA-APPROVED MOLECULAR TEST BASED ON
GENE EXPRESSION PROFILING TECHNOLOGY
FOR BREAST CARCINOMAS**

AGENDIA recommendation criteria :

- **Fresh tissue samples**
- **Sampling not later than 1 hour from the surgical excision**
- Tissue cores must be stored in RNA later.
- Samples must be sent rapidly by courier
- **RIN (RNA Integrity Number) > 6**
- More than 30 % of cancer cell present

**Sixty-six consecutive breast carcinomas were collected under-
vacuum conditions at surgical theatres and kept at 4°C up to sampling**

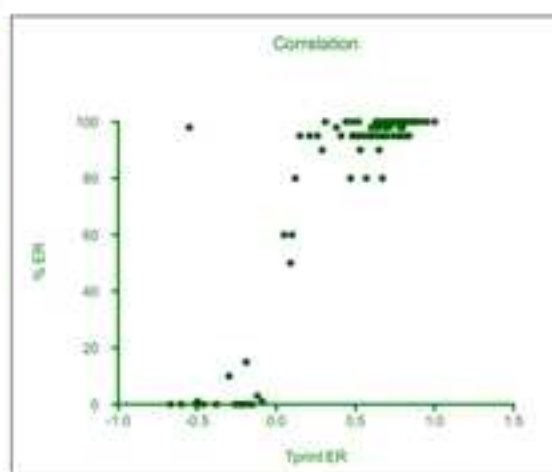
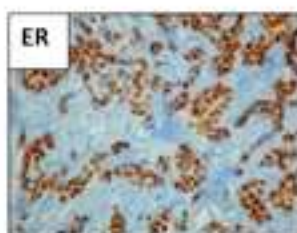




UNDER-VACUUM STORED SAMPLES

Tot cases	Time to Sampling	R.I.N.	MammaPrint Risk	
			Low	High
3	2h	[7,6-8,9]	0	3
7	5h	[7,3-8,3]	3	4
4	6h	[7,3-9,1]	3	1
6	7h	[7,0-9,4]	2	4
2	20h	[7,8-8,5]	1	1
15	24h	[6,8-9,0]	4	11
2	48h	[6,6-8,6]	0	2
6	60h	[7,4-8,1]	5	1
15	72h	[6,2-8,2]	4	11

Spearman's rank correlations: IHC vs Targetprint



	ER %
Number of XY Pairs	116
Spearman r	0.7278
95% confidence interval	0.6296 to 0.8055
P value (two-tailed)	< 0.0001
P value summary	***
Exact or approximate P value?	Gaussian Approximation
Is the correlation significant? (alpha=0.05)	Yes



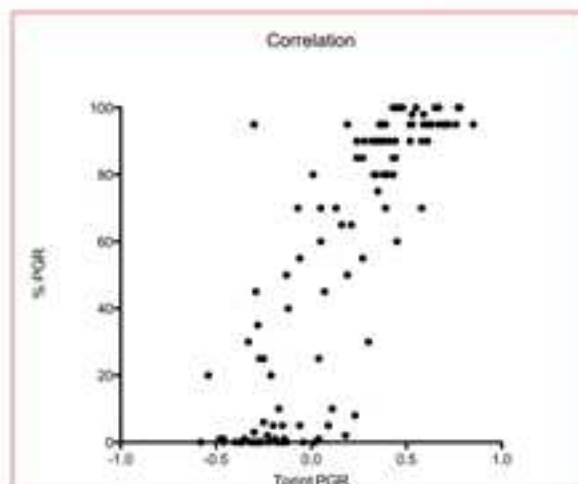
Education and Culture DG

Lifelong Learning Programme

EACEA

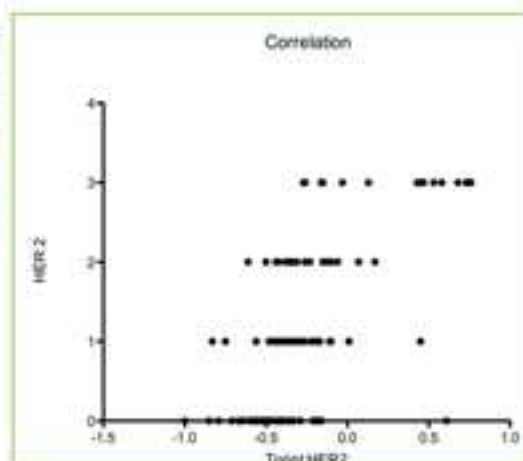
Education, Audiovisual & Culture
Executive Agency

Spearman's rank correlations: IHC vs Targetprint



	PR %
Number of XY Pairs	115
Spearman r	0.8616
95% confidence interval	0.8036 to 0.9034
P value (two-tailed)	< 0.0001
P value summary	***
Exact or approximate P value?	Gaussian Approximation
Is the correlation significant? (alpha=0.05)	Yes

Spearman's rank correlations: IHC vs Targetprint



	HER2
Number of XY Pairs	116
Spearman r	0.6151
95% confidence interval	0.4832 to 0.7197
P value (two-tailed)	< 0.0001
P value summary	***
Exact or approximate P value?	Gaussian Approximation
Is the correlation significant? (alpha=0.05)	Yes



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Conclusion 1.

These results provide evidence on the reliability of the under-vacuum tissue collection in preserving RNA integrity and immunohistochemistry profiling and suggest a way to circumvent feasibility issues related to collection of fresh samples to be analysed by molecular tests.



CELL CULTURES
STEM CELL ISOLATION



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

September 2013 | Volume 8 | Issue 9 | e75193

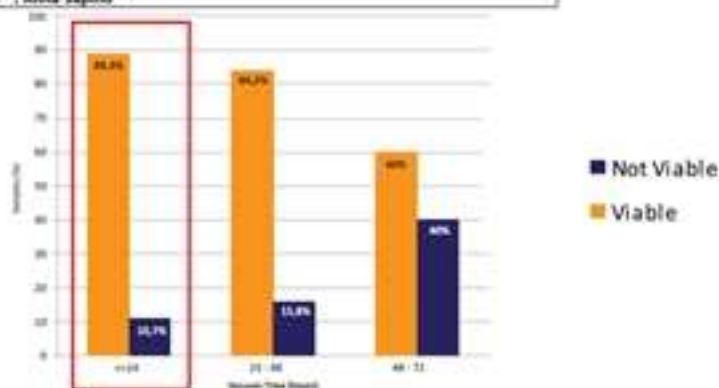
OPEN ACCESS | Freely available online

PLOS ONE

A Collection of Primary Tissue Cultures of Tumors from Vacuum Packed and Cooled Surgical Specimens: A Feasibility Study

Laura Annaratone^{1*}, Caterina Marchisio^{1,2*}, Rosalia Russo¹, Luigi Ciardo¹, Sandra Milena Rondon-Lagos¹, Margherita Gioia¹, Maria Stella Scalzo^{1,2}, Stefania Bolla^{1,2}, Isabella Castellano^{1,2}, Ludovica Verdun di Cantogno¹, Gianni Bussalini^{1,2}, Anna Sapino^{1,2}

52 cases



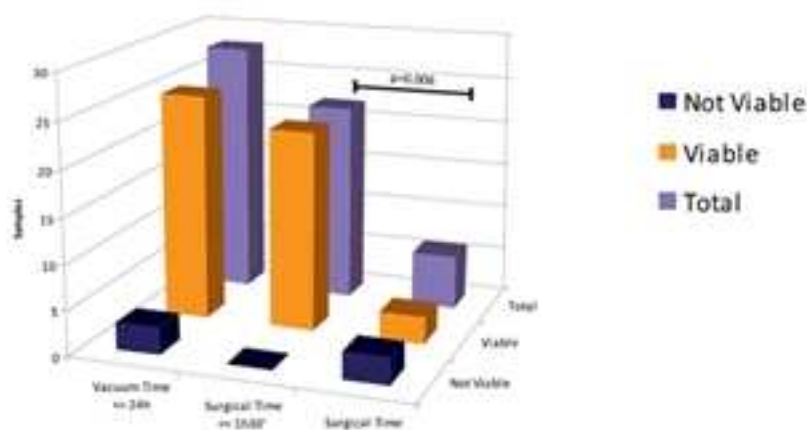
Cell viability (assessed by trypan blue dye -exclusion assay):

89.3% in specimens with Under Vacuum ≤ 24 h

84.2% in specimens with Under Vacuum 24 h-48 h

60% in specimens with Under Vacuum > 48 h.

Assessment of cell viability in specimens with under vacuum time ≤ 24 h and comparison with surgical time.



The difference in cell viability between the two groups (surgical time ≤ 1 hour and 30 minutes and surgical time > 1 hour and 30 minutes) was statistically significant ($p = 0.006$, Fisher's exact test).

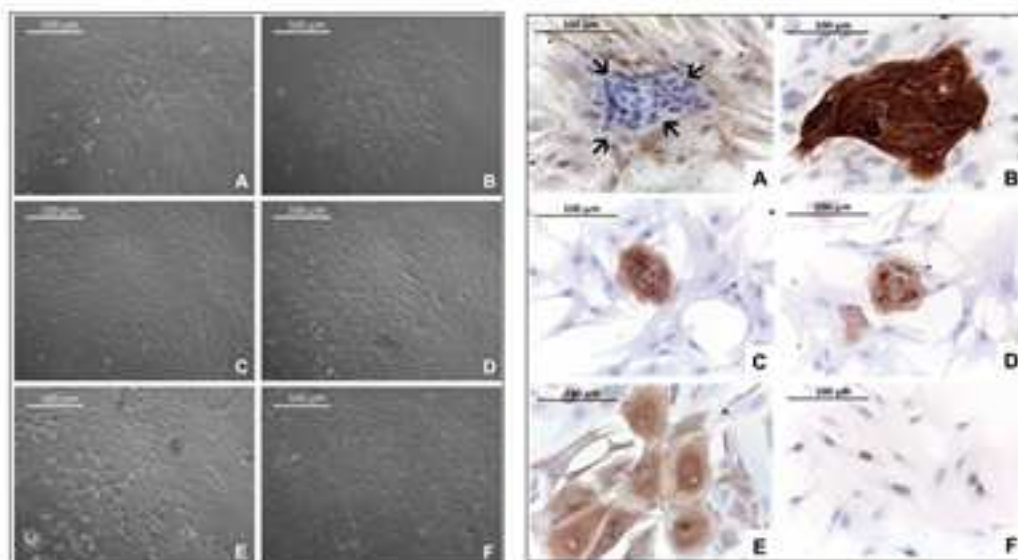


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



A: Breast carcinoma; B: Colorectal carcinoma;
C: Lung adenocarcinoma; D: Gastric
carcinoma; E: Pheochromocytoma; F: Hurtle
cell carcinoma of the thyroid.

A, B: Breast carcinoma: fibroblasts positive for
vimentin (A) and epithelial cells with CK-19
positivity (B).
C, D: Lung adenocarcinoma: epithelial cells
positive for CK-19 (C) and CK-7 (D).
E, F: Hurtle cell carcinoma of the thyroid:
positive for CK-19 (E) and TTF1 (F).

Conclusion 2.

These results provide evidence on the reliability of the under-vacuum tissue for creation of primary cell cultures, showing how a careful monitoring of surgical and under vacuum times fostered a good performance of primary tissue cultures.



Education and Culture DG

Lifelong Learning Programme

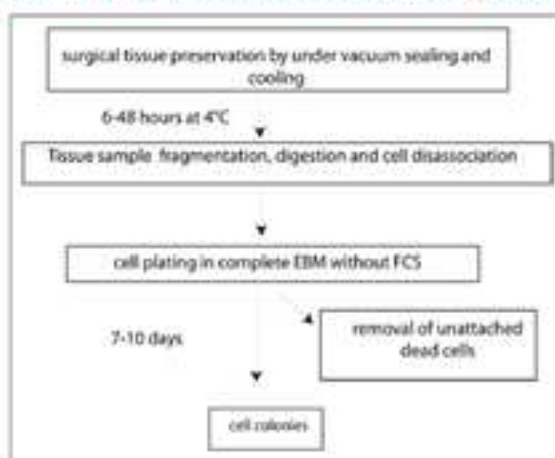


Education, Audiovisual & Culture
Executive Agency



Low oxygen tension is an important component of the stem cell microenvironment.

Hypothesis: the anoxic conditions of tissue samples under vacuum may allow survival of undifferentiated stem/progenitor cells.



SUCCESSFUL ISOLATION OF CD133+ CELLS FROM 20 RENAL TISSUE SAMPLES MAINTAINED UNDER VACUUM AT 4°C.



Morphology of cells isolated from tissues preserved by Under Vacuum after 5 days culture



Morphology of cells isolated from tissues preserved by Under Vacuum after 10 days culture.



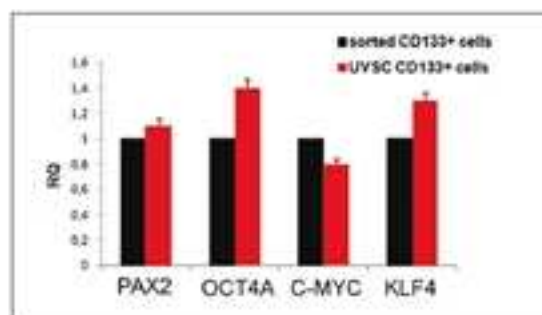
Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Comparison between CD133+ cells from Under Vacuum versus fresh tissue



Quantitative RT-PCR analysis of Under Vacuum CD133+ cells or of CD133+ cells sorted by fresh tissue (sorted CD133+ cells) showing the expression of mRNAs encoding for the renal embryonic and stem-cell related transcription factors.

Conclusion 3.

These results provide evidence on the reliability of the under-vacuum tissue for stem/progenitor cell isolation.

The Under vacuum procedure leads to selection of hypoxia-resistant cells, and therefore may limit the contamination of unwanted cell types or of differentiated cells within the culture.



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



TISSUE BANKING

LETTER TO THE EDITOR

Virchows Arch. 2008 Feb;452(2):229-31.

**Tissue transfer to pathology labs: under vacuum
is the safe alternative to formalin**

Gianni Bussolati • Luigi Chiusa • Antonio Cimino •
Giuseppe D'Armento

Tissue banking

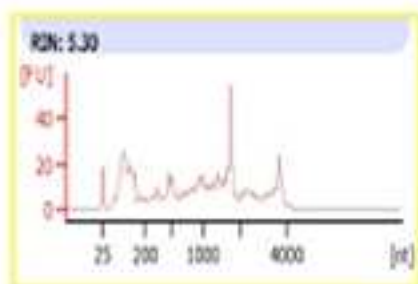
- Written informed consent
- Freezing procedure: the sample is frozen in pre-cooled isopentane immediately after dissection for 15 minutes and stored at -80°C.



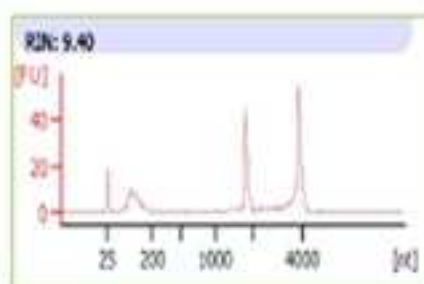
Before under-vacuum



New protocol using under vacuum



Before under-vacuum



New protocol using under vacuum



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Conclusion 4.

These results provide evidence on the reliability of the under-vacuum tissue in performing sampling for frozen tissue biobanking.

Conclusions

- 1) These results provide evidence on the reliability of the under-vacuum tissue collection in preserving RNA integrity and immunohistochemistry profiling and suggest a way to circumvent feasibility issues related to collection of fresh samples to be analysed by molecular tests.
- 2) These results provide evidence on the reliability of the under-vacuum tissue for creation of primary cell cultures, showing how a careful monitoring of surgical and under vacuum times fostered a good performance of primary tissue cultures.

These results provide evidence on the reliability of the under-vacuum tissue for stem/progenitor cell isolation. The Under vacuum procedure leads to selection of hypoxia-resistant cells, and therefore may limit the contamination of unwanted cell types or of differentiated cells within the culture.

- 3) These results provide evidence on the reliability of the under-vacuum tissue in performing sampling for frozen tissue biobanking.



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Take home messages

Under Vacuum collection, preservation and storage of surgical specimens already represented a helpful strategy to bridge diagnostic and experimental pathology

Under Vacuum transfer of surgical tissue increases the number of samples suitable for cell isolation and biobanking, limiting the need of a prompt presence of the cell biologist in the surgical service.



TASTE



Telepathological **AS**essment of histopathological and cytological **TE**chniques

Preeanalytical Issues in Molecular Studies

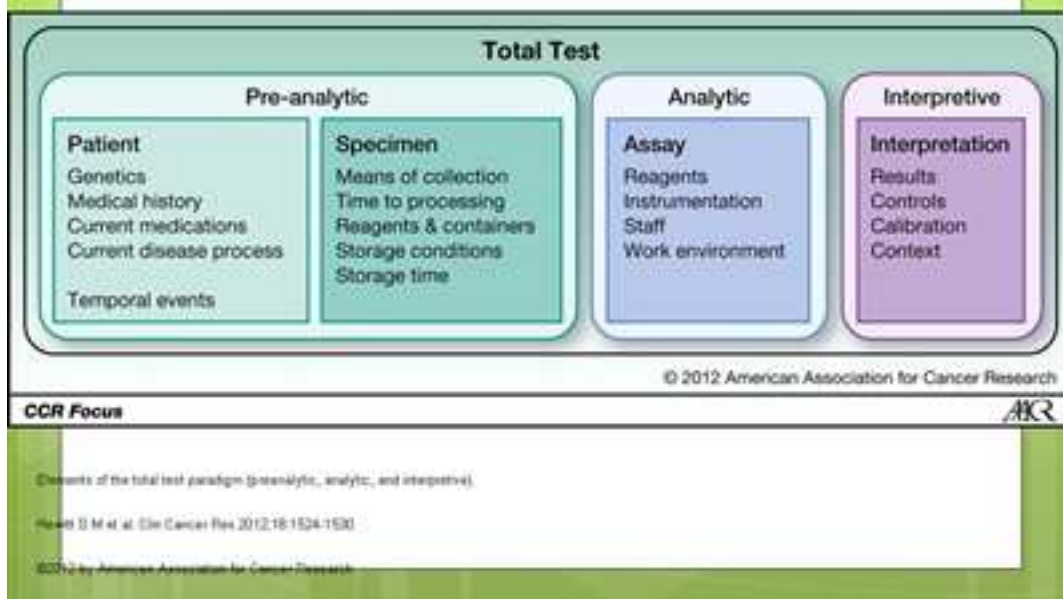
Maria Comanescu

- Preeanalytic variables = those procedures that take place between specimen collection and experimental analysis
- Patient - variables cannot be controlled,
- Collection of the specimen- specification and control
- Avoid Specimen Labeling Errors!!!

Hewitt DM, Talasila M, Braunschweig T, Chung J-Y, Balmori M. *Med* 2007;1313-8



- Specimens - limited use for certain types of research
- Acquisition
- Processing
- Storing
- Clinical data
- Consent?





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

- First step – histology – handling specimens and processing is standardized with regards to molecular biology
- FFPE - fragmentation and chemical modifications in DNA (cross-linking, deamination...)

"tissue was fixed and paraffin-embedded per standard protocol."

- Molecular friendly reagents, but histopathological examination rules!

- Fresh – better but difficult to use in some cases w/o pathology (no tumor, too much normal tissue – dilute total DNA extracted)
- Fine needle – few cells
- Minimum input ???



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

- in situ - specific biologic context - reflected in its molecular profile
- Variables
- Alterations in the molecular profile of the specimen

- state of the specimen at the time it was collected—
- the expression pattern of genes and proteins will depend on both
 - the biological state of the specimen
 - the environmental and biological stresses during the processes of acquisition, processing, storage, and distribution
- *the value of a biospecimen to a researcher is the information it contains about the actual biological state of the specimen as it existed in the person from whom it was derived, determining which changes are disease-related and which are artifacts*

RAND study



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

- same genes w/ different expression in diseases, but also in response to environmental factors
- significant changes in gene expression can occur as early as 15 minutes after exposure to a stimulus or stress, while posttranslational changes in proteins, such as methylation and phosphorylation, can occur within seconds

Laboratory testing



Biological variability
Environmental variables
Patient's identification
Patient's preparation
Sample collection device
Sample collection procedure
Container
Sample handling
Sample separation
Sample storage

Lepp et al. - Preanalytical variability and laboratory testing



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



Transportation, Space, and Technology
A RAND INFRASTRUCTURE, SAFETY, AND ENVIRONMENT PROGRAM

Acquisition:

- Time of ischemia (warm, cold)
- Grossing by the pathologist
- Processing – in the molecular biology laboratory:
 - isolation, purification, fixation, and preservation
 - time at room temperature, temperature of room, type of fixative, time in fixative, method and rate of freezing, and size of specimen aliquots

RAND study



Storage:

- storage temperature, duration of storage, and progressive dehydration, desiccation, or oxidation
- distribution: transport conditions (e.g., shipped in liquid nitrogen, on dry ice, or at ambient temperature).

RAND study

- In molecular pathology we are interested in changes in:
 - DNA,
 - RNA,
 - Protein.
- altered by exposure to preanalytical variables.
- depending on what we are looking for → choose a certain method/ technological platform



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

DNA - most robust

- **Quality - length of DNA fragments**
- **Does the length of the DNA affects assay performance?**
- **Array comparative genomic hybridization (aCGH)** - copy number variations - DNA fragment length affects call rates – alteration of cutoffs for the presence of a genomic alteration. shorter DNA fragments = less information indefining small regions of loss or gain

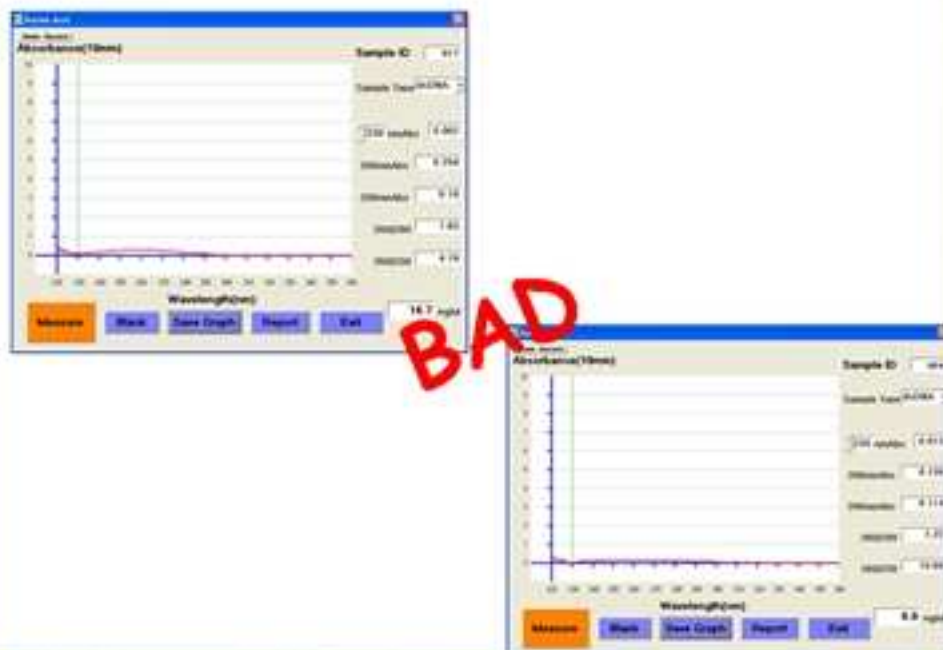
DNA - most robust

- **Sequencing - false-positive mutations - because of alterations (chemical crosslinking)**
- **electrophoresis,**
- **FISH**
- **PCR**
- **SNP**



Quantification and qualification of DNA samples by NanoDrop

- DNA quantity and quality measured by reading the whole absorption spectrum (220–750 nm) with NanoDrop
- DNA concentration and absorbance ratio at both 260/280 and 230/260 nm



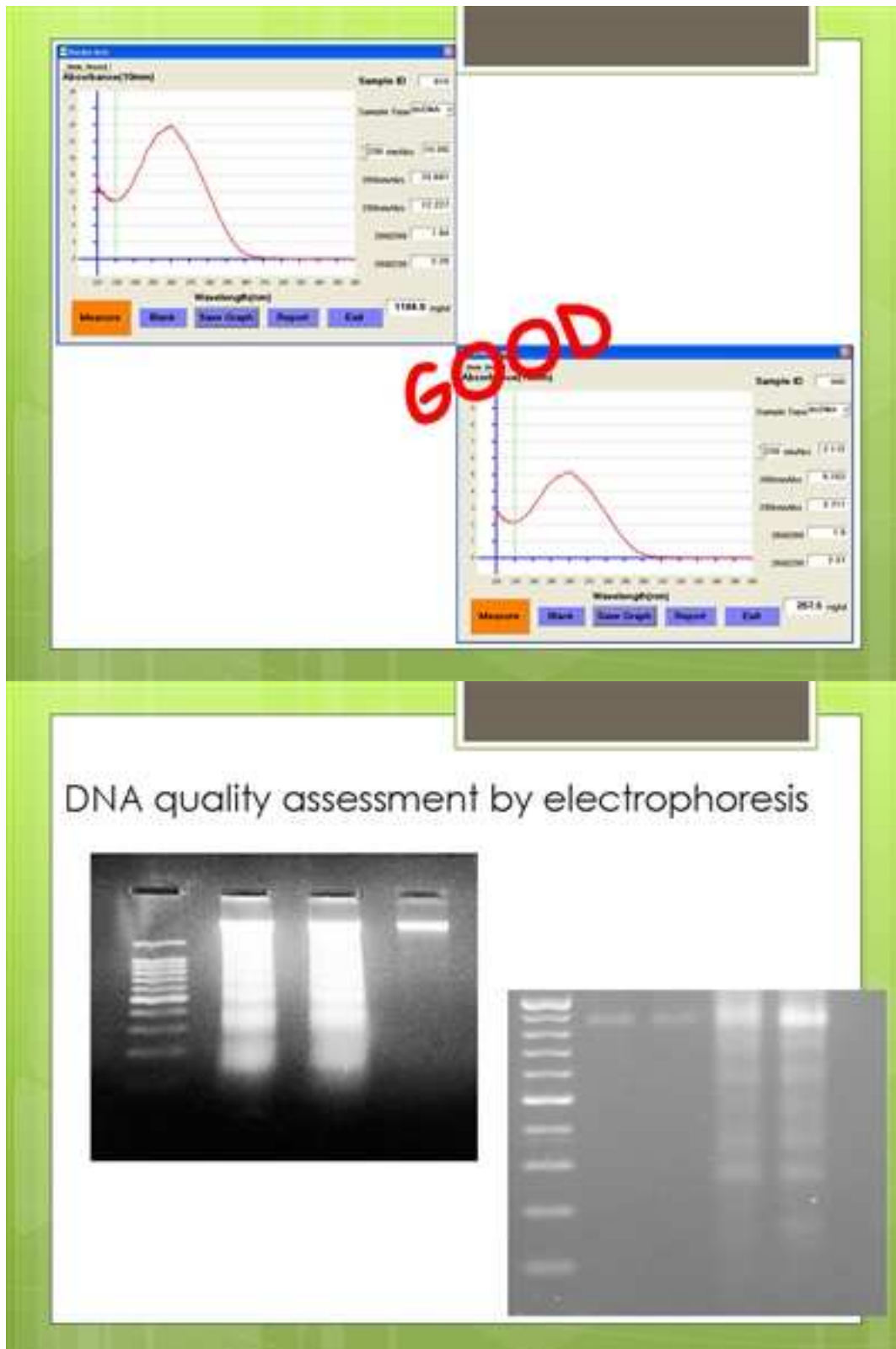


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

RNA

- RNA can be isolated from formalin- fixed, paraffin- embedded (FFPE) tissue
- RNA quantity and quality are significant issues in assay development
- substantial variation in RNA quality (total RNA, specific mRNA)
 - fixation time (both under and overfixation),
 - processing (longer time – improved preservation – better extraction of water)
 - embedding

Chung JY, Braunschweig T, Williams R, Guemero N, Hoffmann RM, Kwon M, et al. / *Histochem Cytochem* 2008;56:1033–42

RNA – most labile

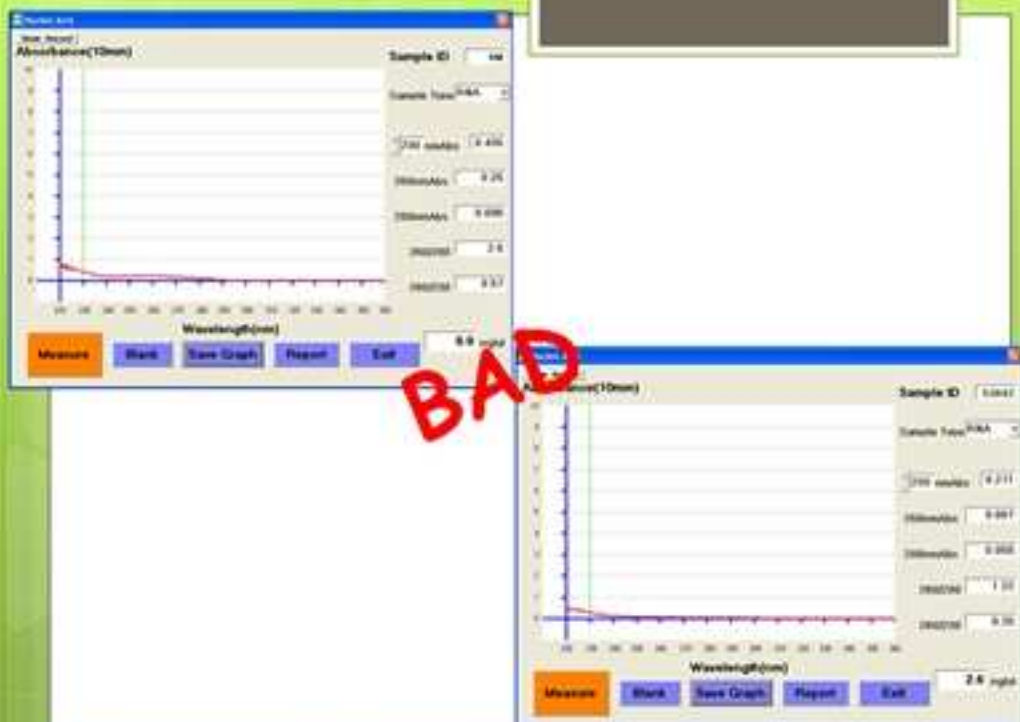
- mRNAs are encoded with multiple elements that impact the stability of RNA
- RNA measurement – PCR (amplicon size) and hybridization techniques (probe)
- Preanalytic conditions – identification (Y/N)
- Quantitative RT-PCR very dependent on the quality of the starting RNA – no DENIED DENOMINATOR OF QUALITY
- MORE control genes

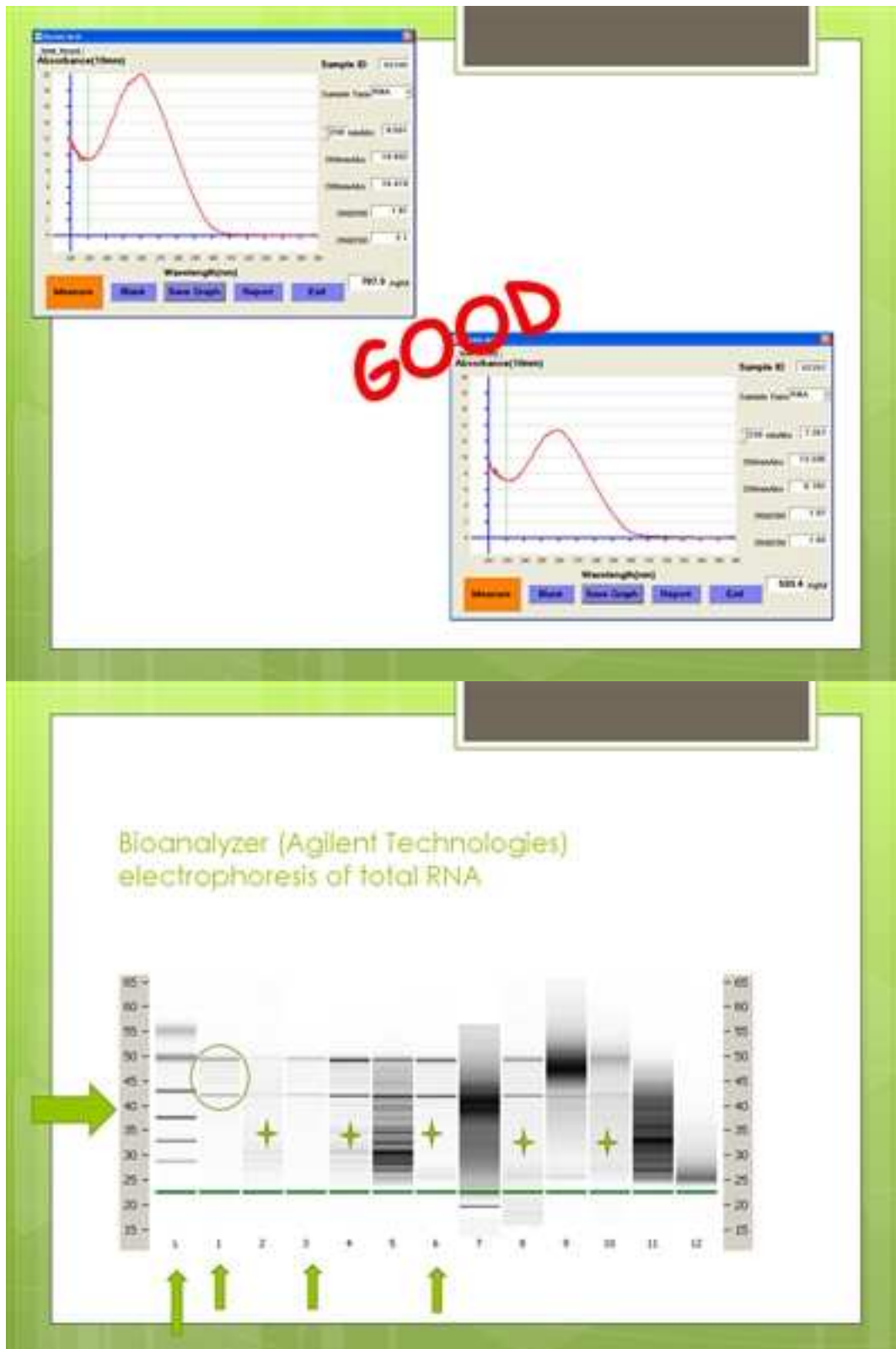




RNA - most labile

- cDNA microarrays
- ISH
- electrophoresis
- RT-PCR
- real-time PCR







Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

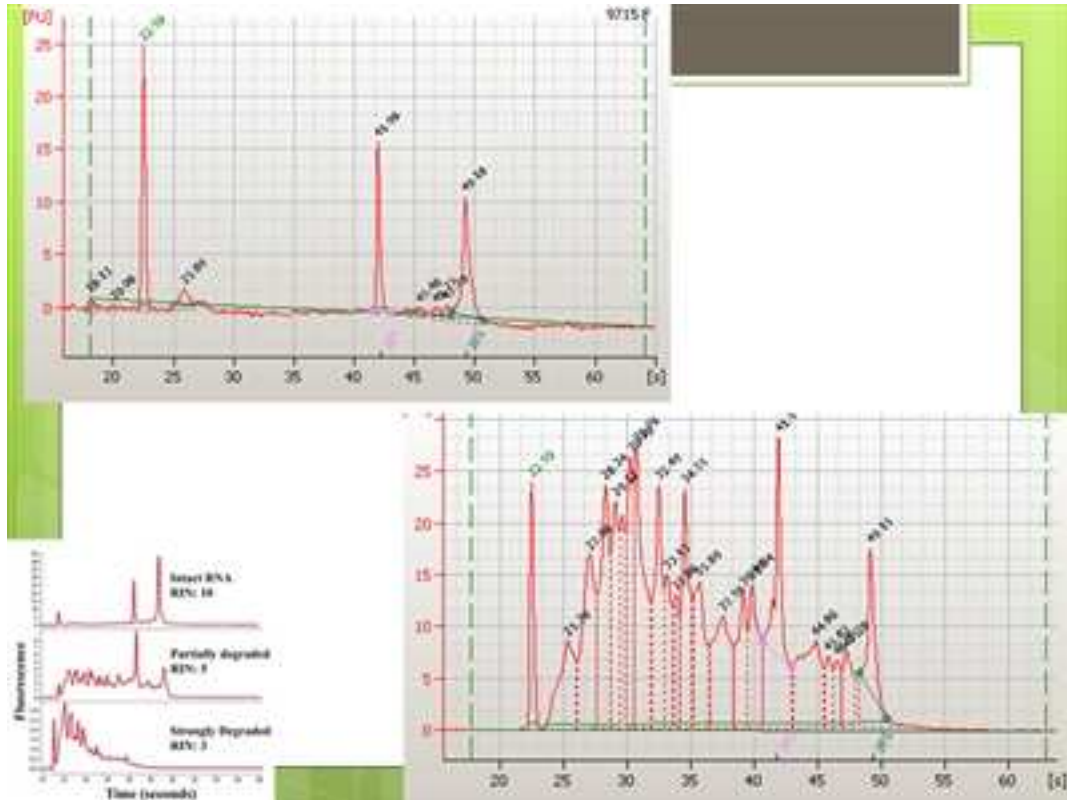
Goldilocks Principle



- spectrophotometric methods to assess RNA quality
- run agarose gels.
- Agilent 2100 bioanalyzer from Agilent Technologies Inc.-
 - the integrity of the ribosomal bands on the mini gel-like image or
 - the ribosomal peak ratio on the electropherogram

RIN

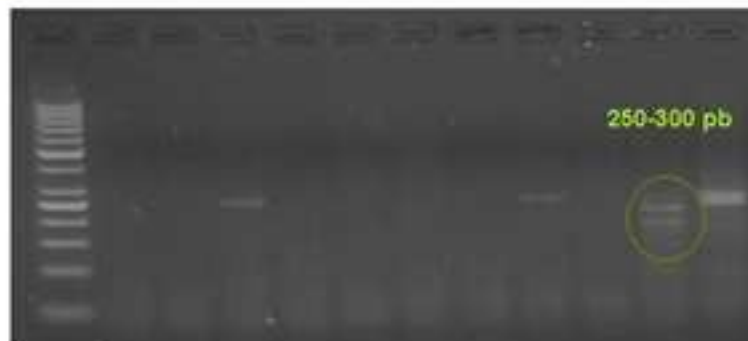
- The **RNA integrity number** (RIN) is an algorithm for assigning integrity values to **RNA** measurements.
- Estimating the integrity of RNA samples and the ratio of 28S:18S ribosomal RNA





Rearrangement study of IgH in B lymphomas

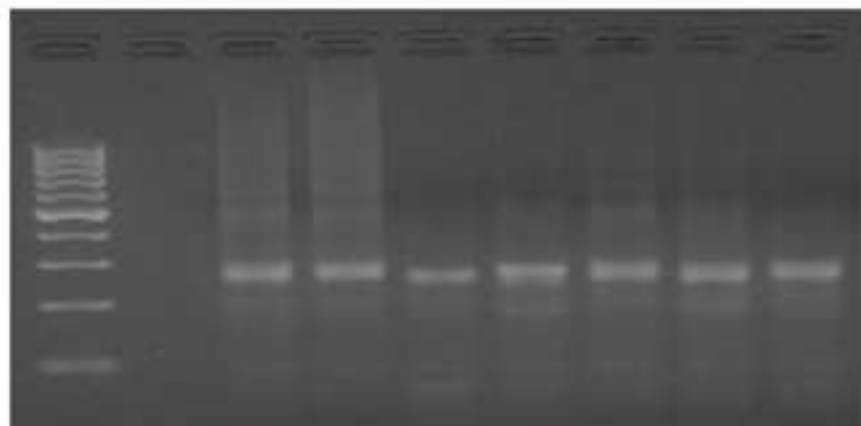
L NTC 1 2 3 4 5 6 7 8 9 10



1, 3, 4, 5, 6, 8 – no amplification
2, 7, 9, 10 – w/ amplification

L – 100 bp DNA Ladder; NTC – No Template Control; 1, 2, 3, 4, 5, 6, 7 – w/ amplification

L NTC 1 2 3 4 5 6 7





Education and Culture DG

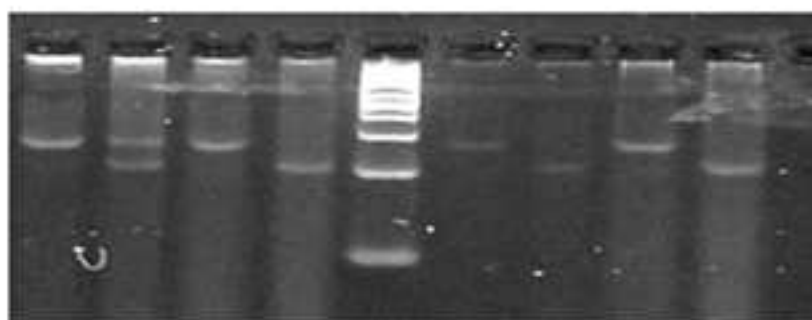
Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

KRAS – codon 12

PC NC L 1 2



PC – positive control, NC – negative control, L – 50 bp DNA Ladder, 1 – low amplification, 2 – w/ amplification

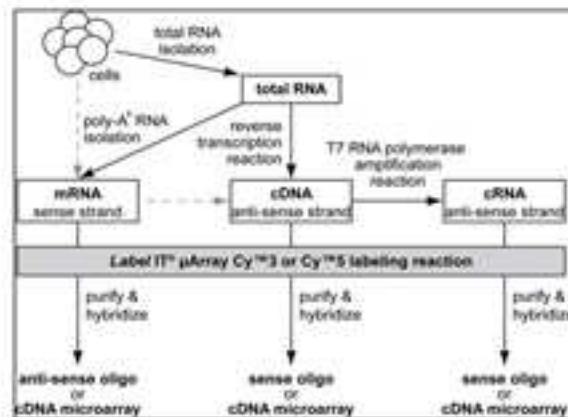
primers configuration and amplification reaction
– more sensible

Kras codone 13

PC NC L 1 2



Cy3 Labeling of cDNA Samples



Mirus

NanoDrop ACTGene ASP-3700 (ACTGene)

- quantity of labeled cRNA (UV, 260nm and 550nm)
- cRNA quantity
 - $(\text{cRNA concentration}) \times 30 \mu\text{l (elution volume)} / 1000 = \mu\text{g cRNA}$, minimum 1,65 μg
- Specific activity
 - $(\text{Cy3 concentration}) / (\text{cRNA concentration}) \times 1000 = \text{pmol Cy3 per } \mu\text{g of cRNA}$. Minimum 6 pmol Cy3 per μg



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

The image is a composite of two parts. The top part shows two screenshots of a software interface titled 'New Result'. Both screenshots display a graph of Absorbance vs. Wavelength (nm) with a peak around 260 nm. The left screenshot is labeled 'GOOD' in large red letters, and the right screenshot is labeled 'BAD' in large red letters. The bottom part is a three-panel comic strip. The first panel shows a building labeled 'LABORATORIES' with a speech bubble saying 'AAAGHHH!!'. The second panel shows a doctor at a desk with a speech bubble saying 'DOCTOR! ONE OF THE LAB TECHS IS HAVING A HEART ATTACK!'. The third panel shows the doctor at the desk with a speech bubble saying 'QUICK! WE NEED TO GIVE HIM PCR!'.



Lifelong Learning Programme



Pre-analytical issues
and
Liquid-Based Cytology
Francesco Feoli, Institut Jules Bordet, Brussels,
Belgium



The Third TASTE Workshop
PRE-ANALYTICAL ISSUES IN PATHOLOGY
Ponte, Portugal, 19th October 2013

Institute for Pathologists
in Clinical Cytopathology

Johns Hopkins University
School of Medicine
Baltimore, MD

**Cytopathology is a *Medical Diagnostic Technique*
Based on the Study
of *Micro Biopsies* (Cellular Samples).**

1984





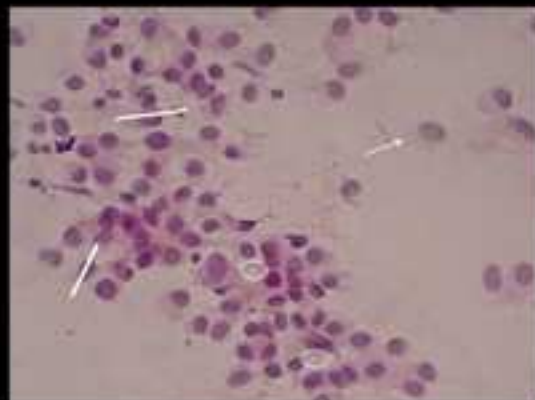
Cellular samples are *Micro Biopsies*



Cytopreparation:

- Concentration
- Purification
- Spreading
- Fixation
- Staining
- Mounting
- Dotting

Study of *Micro Biopsies* (Cellular Samples)



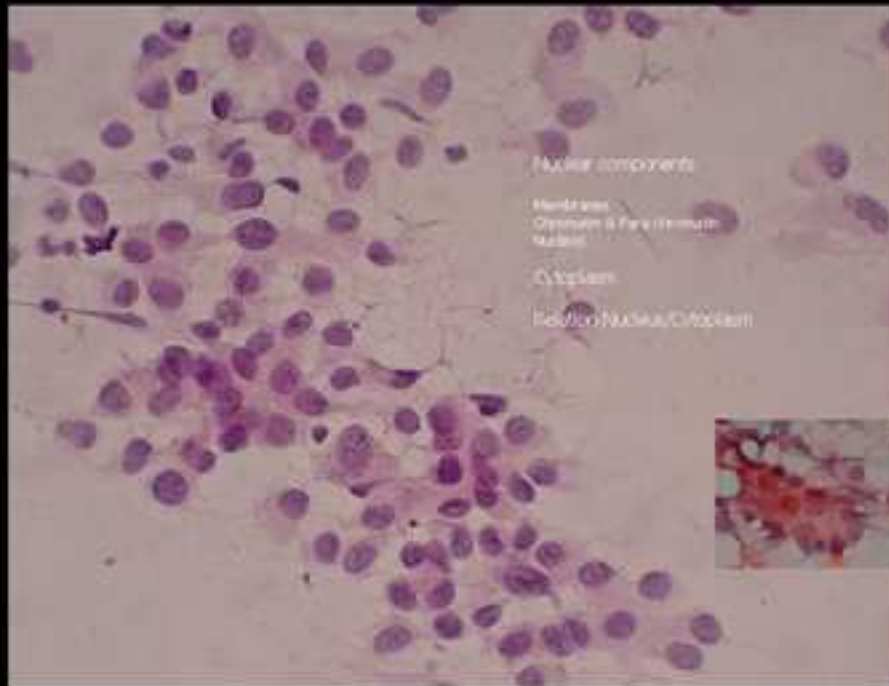


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Nuclear components

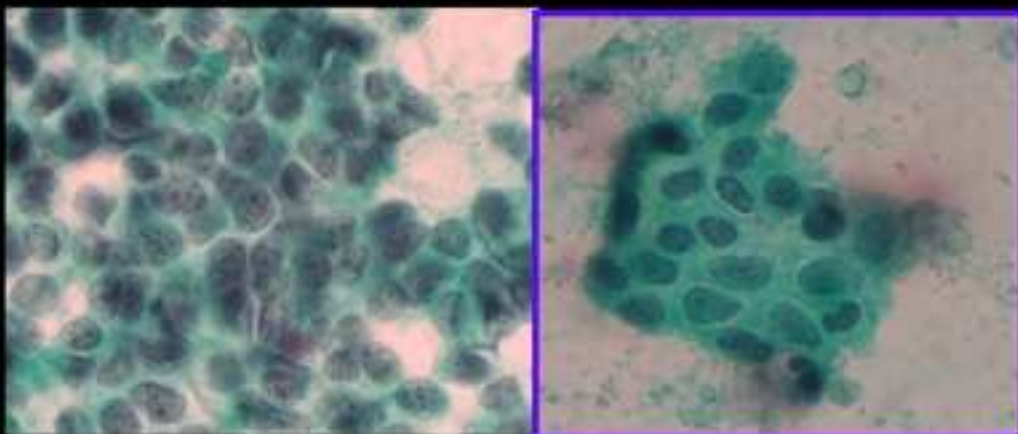
Membrane
Chromatin & Fine structure
Nucleolus

Cytoplasm

Reddened Nucleus/Cytoplasm

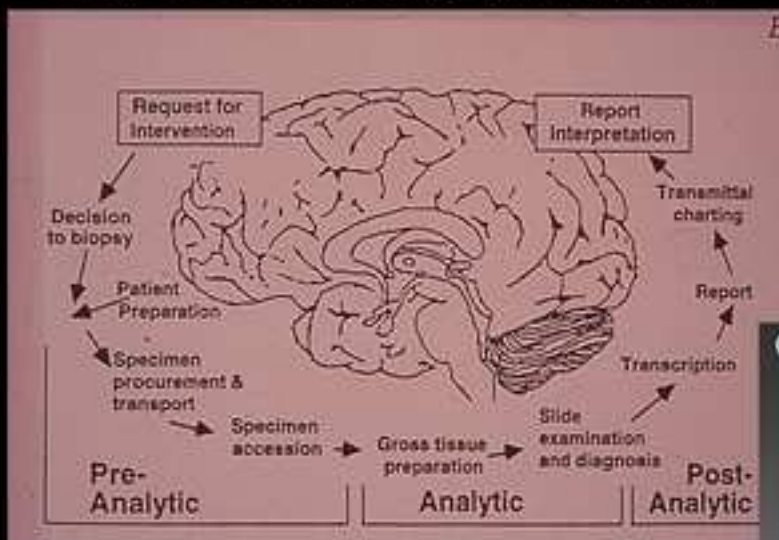
Alcohol Fixed Material & Papanicolaou Staining:
Improves Accuracy
Reduces Uncertain Diagnoses

F. Feoli, M.D., et al. Acta Cytol 2008 ; 52:145-151.





Study of *Micro Biopsies* (Cellular Samples)



Cytopreparation:

Concentration
Purification
Spreading
Fixation
Staining
Mounting
Dotting

The Third TASTE Workshop
PRE-ANALYTICAL ISSUES IN PATHOLOGY
Porto, Portugal, 19th October 2013

Exceptions (Pre Analytical Phase)

The screenshot shows the website of the Papanicolaou Society of Cytopathology. It features a navigation menu on the left with links to 'ABOUT US', 'NEWS', 'CASE OF THE MONTH', 'FOOD GUIDELINES', 'AWARDS', and 'LINKS'. The main content area displays a banner for a DVD by Britt-Marie Ljung, M.D., UCSF, titled 'PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY'. Below the banner, there are four microscopic images of cellular samples. A large image of a 'Breast FNA' (Fine Needle Aspiration) sample is shown on the left. On the right, a list of topics for the DVD is provided:

- Expulsion Onto Slide
- Flip Technique
- Basic Smearing Technique
- Dividing Material
- Problem Material
- Special Problems

The website URL <http://www.papsociety.org> is visible at the bottom right.



Education and Culture DG

Lifelong Learning Programme

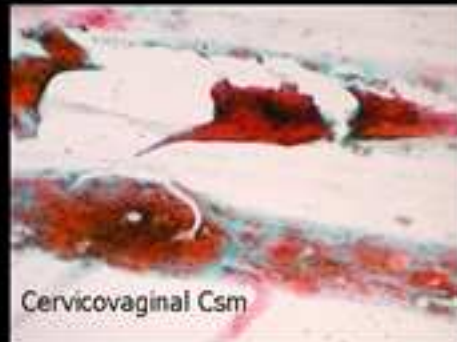


Education, Audiovisual & Culture
Executive Agency

Exceptions (Pre Analytical Phase)



Breast FNA



Cervicovaginal Csm



Cervicovaginal Csm

A. Cervicovaginal Cytology



1987
Wall Street Journal Nov 2, 1987



Toward Optimal Laboratory Use



Developed by coordinating ASCP, American College of

Cytology (ACCP) and CAP

Journal of Clinical Microbiology

The 1988 Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses

Developed by the International Association of

American Society of Clinical Pathologists
College of American Pathologists

SUMMARY


Medicare, Medicaid, and CLIA Programs

Regulations Implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88)


Proposed Rule

May 21, 1990


42 CFR Parts 405, 404, 406, 402, 403, 408, and 409



Cervicovaginal Cytology



Obstet Gynecol. 2008 Jan;111(1):14-22.



Liquid Compared With Conventional Cervical Cytology

A Systematic Review and Meta-analysis

Mark J. Arora, MD, PhD, Christine Bergman, MD, PhD, Paul K. Kulkarni, MD, PhD, Maria Martin-Miranda, MD, PhD, Alberto G. Suarez, MD, and John A. Bello, MD, PhD

LBC:
Reduction of the Inadequate Samples.
UK: 9-11% to 2%



Cervicovaginal Cytology

Improvements

Pre analytical phase



Lancet Oncol. 2010 Mar;11(3):249-57; Epub 2010 Jan 18.

Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial.

Ronco G, Giorgi-Rossi P, Carozzi F, Confortini M, Della Palma P, Del Mistro A, Ghiringhella B, Girlando S, Gilio-Tos A, De Marco L, Naldoni C, Pierotti P, Rizzolo R, Schiavaglia P, Zorzi M, Zappa M, Segnan N, Cuzick J, New Technologies for Cervical Cancer screening (NTCC) Working Group.

Post analytical phase

LBC and Artifacts

Focal point slight profiler

Range Found 12.12 - 09.13.

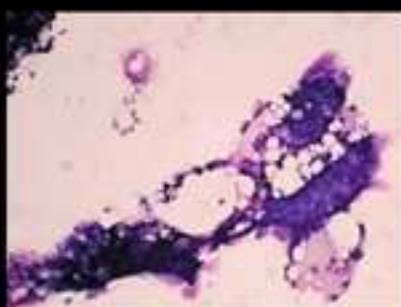
All Slides	7981
Rerun	32 (0,4%)
Process Review	922 (11.5%) 2% NL
Scant cellularity	302 (3,8%)





B. FNA Cytology

Feoli F et al. Acta Cytol 2013; 57:369-376.



LBC reduces air-drying, spreading artifacts, obscuring effects of blood and inflammation.

In small community practices with low workloads [20].

In large establishments with large numbers of practitioners of varying levels of experience [10].



Feoli F et al 2013; 57:369-376.

Retrospective Revue of 190 LBC Cases With Paired Biopsies. No Clinical Information.

	Suggested Acceptable Values	Readings			P-value		
		FF1 (No Training)	FF+PVE (Collaboration) (No Training)	FF2 (Post Training)	FF1 vs. FF+PVE	FF1 vs. FF2	FF+PVE vs. FF2
Absolute sensitivity: Malignant/2 malignant cases	>60%	55% (88/159)	57% (90/159)	63% (99/159)	0.48	0.72	1
Complete sensitivity: "Positive" (C3+C4+C5)/malignant cases	>80%	66% (117/179)	69% (142/207)	94% (149/159)	0.20	0.001	0.006
Specificity: Benign/2 benign cases	>60%	71% (22/31)	68% (21/31)	63% (20/31)	0.56	0.43	0.56
Suspicious rate: OC3/OC4/total number of cases	<20%	27% (51/190)	29% (56/190)	33% (64/190)	0.30	0.03	0.03
Indeterminate rate: C1/total	<25%	3% (9/190)	3% (9/190)	4% (7/190)	1	0.16	0.16
FP rate: Benign/5-malignant cases	<3%	0.6% (1/159)	0.6% (1/159)	0% (0/159)			
FN rate: Malignant/2-malignant cases	<5%	12% (19/159)	9% (13/159)	4% (7/159)	0.11	0.003	0.03



Wang H.H. *Acta Cytol* 1998; 42:265-268

Feddi F. *Acta Cytol* 2008

Reporting Categories:

C1 Inadequate: n.d.

C2 Benign: PPV 13.8%

C3 Atypia, Probably Benign: PPV 35%

C4 Suspicious: PPV 93%

C5 Malignant: PPV 100%



Four Features



	Suggested Acceptable Values ¹	Accuracy					
		RA		RA, new criteria 1		RA, new criteria 2	
Absolute sensitivity: #malignant/Σmalignant cases	≥60%	* 37%	(96/159)	68%	(108/159)	* 74%	(118/159)
Complete sensitivity: malignant/(C3+C4+C5)/malignant cases	≥88%	93%	(151/159)	95%	(151/159)	98%	(153/159)
Specificity: #benign/Σbenign cases	≥60%	54%	(23/51)	54%	(23/51)	54%	(23/51)
Suspicious rate: (C3+C4)/total number of cases	≤28%	* 34%	(65/190)	25%	(47/190)	* 19%	(36/190)
Inadequate rate: C1/total	≤25%	4%	(7/190)	4%	(7/190)	4%	(7/190)
FP rate: #benign/Σmalignant cases	≤3%	0%	(0/159)	0%	(0/159)	* 0.6%	(1/159)
FN rate: #malignant/Σmalignant cases	≤3%	3%	(5/159)	3%	(5/159)	3%	(5/159)

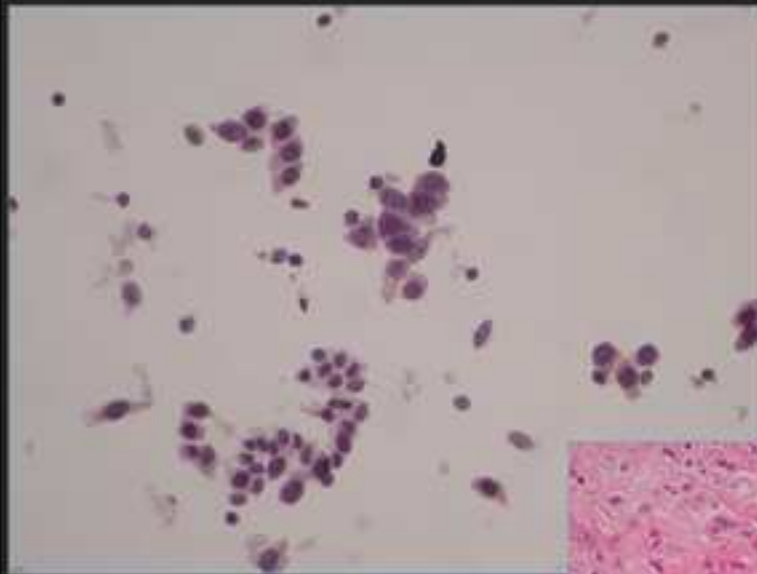


Education and Culture DG

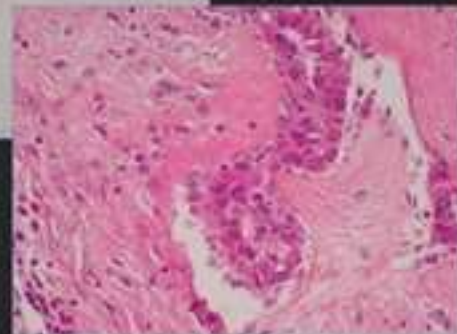
Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Fibroadenoma



Study of *Micro Biopsies* (Cellular Samples)

FNA Cytology of The Breast

LBC Is Not Necessary

In Situations Where the Smears Are
Chronically Suboptimal

It Reduces the Insatisfactory samples
And
May Increase Accuracy



PRE ANALYTICAL PHASE

Integration of Clinical and Morphological Data



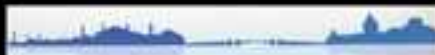
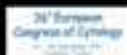
341 Institutions.
771.475 Cases.

0.73% Required Additional Information.

Diagnosis: Changed in 6%.

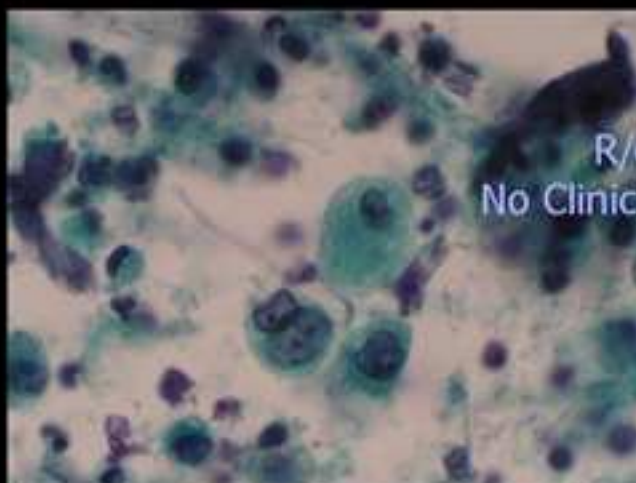
Endoscopic Biopsies 7.4%.

Therapy Induced Change.
Malignant Neoplasm
Inflammation



SLIDE SEMINAR 12
BOSPHORUS Session / Our Mistakes in Cytology...

Alain Verhest / Francesco Feoli, Belgium



BAL
R/o Cancer
No Clinical Information

Table Page 67



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency



Changes in epithelial cells simulating those encountered in cancer of the lung occur in sputum and bronchial washings and brushings, and they are sometimes seen in specimens obtained by fine-needle aspiration.

It is rarely possible to determine the nature of the inciting factor without clinical information.



PRE ANALYTICAL PHASE
Specimen Collection Method



URINE & Bladder

- Voided
- Catheterized
- Washing

UPPER TRACT





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Pre-analytical issues and TMA Construction

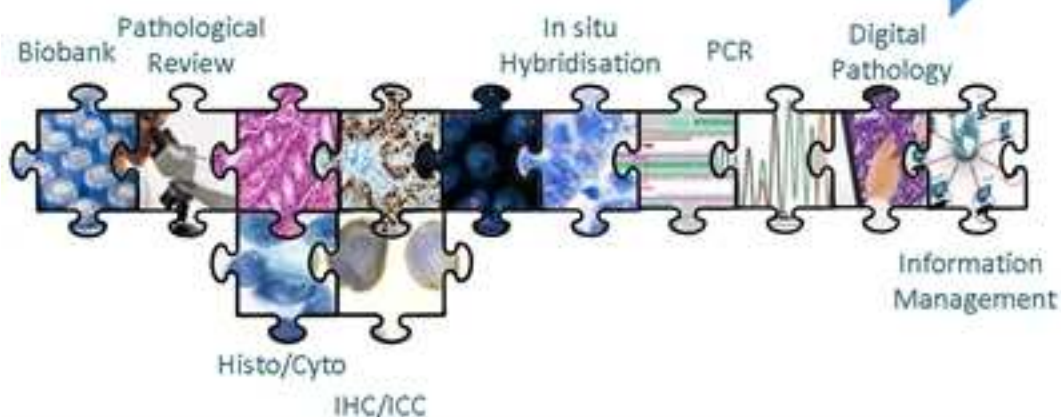


Prof Manuel Salto-Tellez
Chair of Molecular Pathology
Deputy Director, CCRBC
Visiting Professor, National University of Singapore

Porto, October 2013

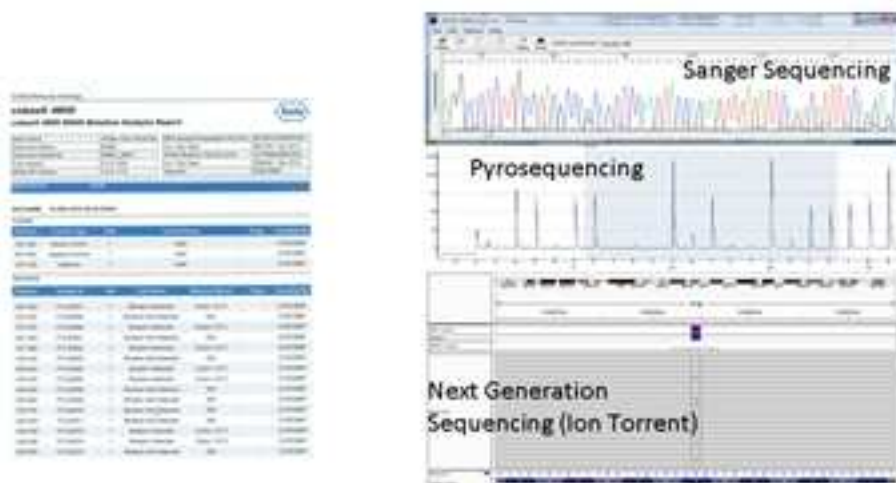
Northern Ireland Molecular Pathology Laboratory (NI-MPL)

End to End Diagnostic and Research Service
Cutting Across Technologies and Infrastructures





KRAS Mutation Analysis



Molecular Pathology: A Practical Guide for the Surgical
 Pathologist and Cytopathologist
 Chapter 15. Tumors of the gastrointestinal system.
 Salto-Tellez, Yan, Wu and Pitman 2013, *In press*.



Next-generation sequencing

OPEN ACCESS Freely available online

PLOS ONE

Validation of Next Generation Sequencing Technologies in Comparison to Current Diagnostic Gold Standards for *BRAF*, *EGFR* and *KRAS* Mutational Analysis

Clare M. McCourt¹, Darragh G. McArt¹, Ken Mills², Mark A. Catherwood^{2,4}, Perry Maxwell^{1,4},
 David J. Waugh³, Peter Hamilton¹, Joe M. O'Sullivan^{3,4}, Manuel Salto-Tellez^{1,3,4,5}

PLOS ONE 8(7): e69604. doi:10.1371/journal.pone.0069604





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

Pre-analytical issues and TMA Construction



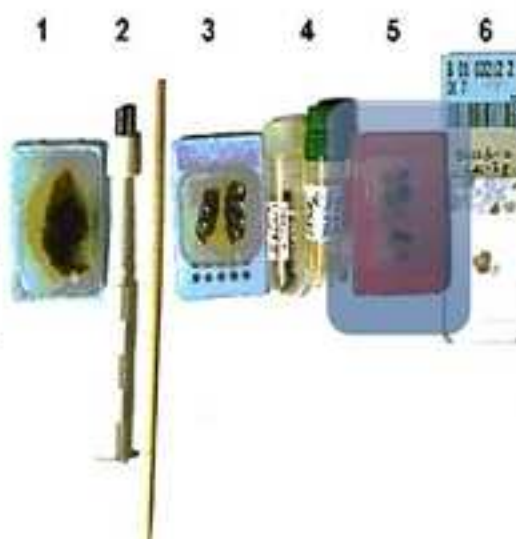
Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

The multitumor ("sausage")
tissue block was
introduced by Hector
Battifora (1986) as a novel
method of testing
monoclonal antibodies for
immunohistochemistry



Queen's University
Belfast

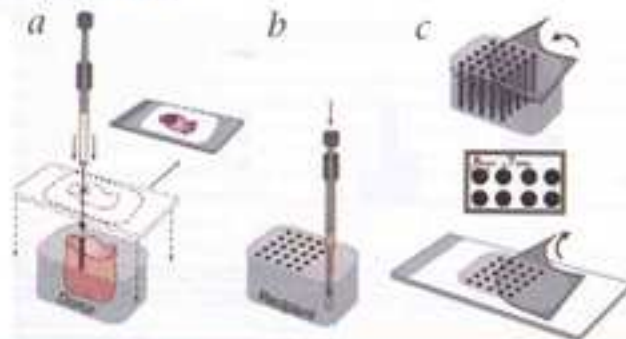
THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Tissue Microarray
Kononen et al. Nat Med. 1998
Jul;4(7):844-7.



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



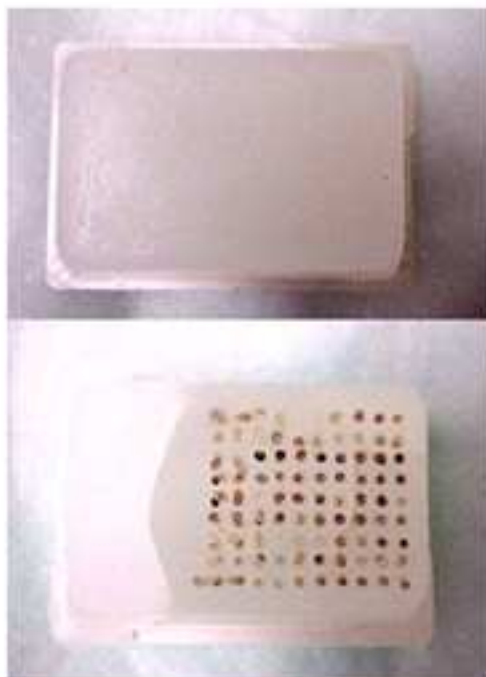
Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust





THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Reliability of Tissue Microarrays in Detecting Protein Expression and Gene Amplification in Breast Cancer

Danilua Zhang, Ph.D., Michael Sabin-Tyler, M.D., Thomas Clouston-Patt, M.D., Sheng Hu, B.Sc.,
Profess Nore-Chuan Kuo, Ph.D.

Mod Pathol 2003;16(1):79-85

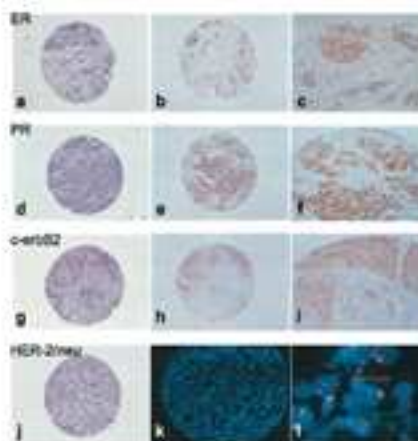


TABLE 1. Comparison between Full Sections and Arrayed Sections of Estrogen Receptor (ER), Progesterone Receptor (PR), c-erbB2, and HER-2/neu Status in Breast Tumors

		Full Sections	Arrayed Sections	Concordance (%)	p-value*
ER	+	56	53	97 (n = 93)	0.33
	-	37	40		
PR	+	56	54	98 (n = 93)	0.35
	-	37	39		
c-erbB2	+	33	30	97 (n = 62)	0.52
	-	59	62		
HER-2/neu	+	23	20	95 (n = 82)	0.88
	-	64	62		



THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Since 2003:

More than 50 articles with a direct use of TMAs
Guidelines for TMA Construction

Histopathology

Histopathology 2013; 62(5): 827-839

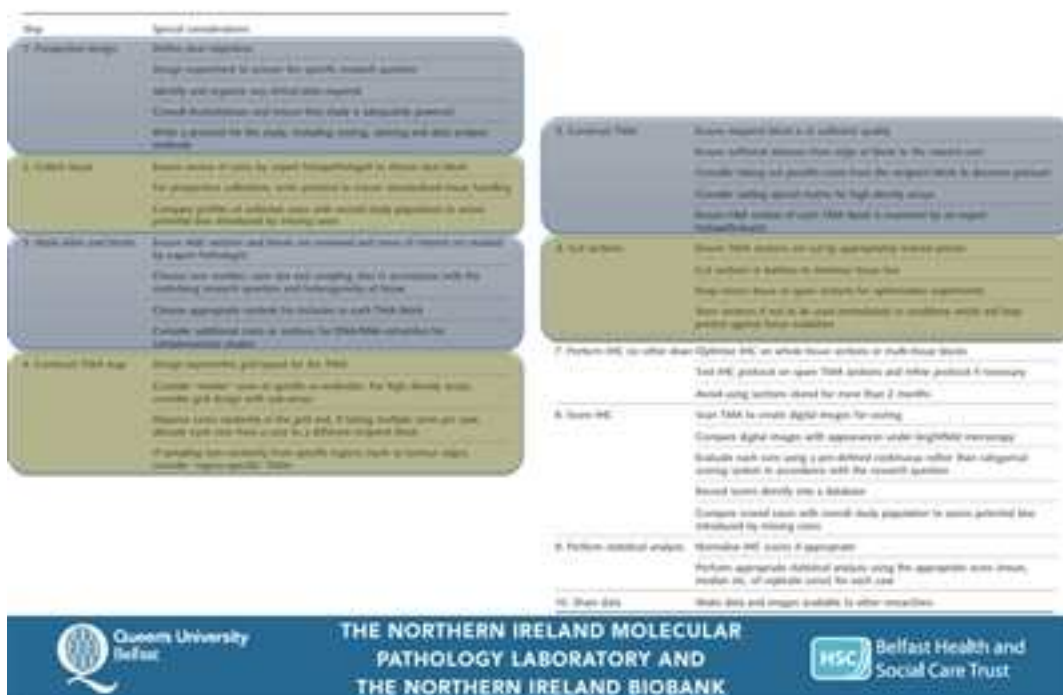
REVIEW

Guidelines and considerations for conducting experiments using tissue microarrays

Mohammad Riyas,¹ Heiko Grubich,² Ian O'Ellis,² Chris Wernack,^{2,4} Robert Brown,⁵
 Dan Bernes,⁶ Dean Tennyson,⁷ Mansur Salto-Telles,⁸ Martin Jenkins,⁹ Stefan Landberg,¹
 Richard Byers,¹ Darren Treanor,¹⁰ David Harrison,¹⁰ Andrew R Green,⁹ Graham Ball¹¹
 & Peter Hamilton^{12,13}



Histopathology. 2013 May;62(6):827-39.





Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

1. Prospective design	Define clear objectives
	Design experiment to answer the specific research question
	Identify and organise any clinical data required
	Consult biostatistician and ensure that study is adequately powered
	Write a protocol for the study, including scoring, staining and data analysis methods

Research plan, database, adequate numbers, analytical protocol



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



NUS
National University of
Science and Technology

Tissue Microarray sets

CRC	RCC	Lymphoma
Gastric Ca	Ovarian Ca	General
HCC	DermPath (skin multiple)	Lung (small)
Breast Ca	BCC	Lung (big)
Ovarian Ca	NPC	Prostate
Sarcoma (translocation-related)	Medulloblastoma	
SarcPath (sarcoma multiple)	Gastritis-IM-Dysplasia-GC	



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency



Tissue Microarray sets

GENERAL SCREENING ARRAY

CRC	RCC	Lymphoma
Gastric Ca	Ovarian Ca	General
HCC	DermPath (skin multiple)	Lung (small)
Breast Ca	BCC	Lung (big)
Ovarian Ca	NPC	Prostate
Sarcoma (translocation-related)	Medulloblastoma	
SarcPath (sarcoma multiple)	Gastritis-IM-Dysplasia-GC	



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

Dermatological malignancies

Type	Name	
Melanocytic	Malignant melanoma	
Squamous	Seborrheic keratosis	Bowen's disease
	Keratoacanthoma	Squamous cell carcinoma
	Actinic keratosis	
Sebaceous	Sebaceous hyperplasia	Poroma
	Sebaceous adenoma	Porocarcinoma
	Sebaceous carcinoma	
Fibroblastic	Dermatofibroma	Dermatofibrosarcoma protuberans
Neural	Neurofibromatosis	Malignant peripheral nerve sheath tumor
	Schwannoma	
Vascular	Pyogenic granuloma	Angiosarcoma
Appendage	Tripitthelioma	Pilomatricoma
	Trichoblastoma	Naevi
	Basal cell carcinoma	



Queen's University
Belfast

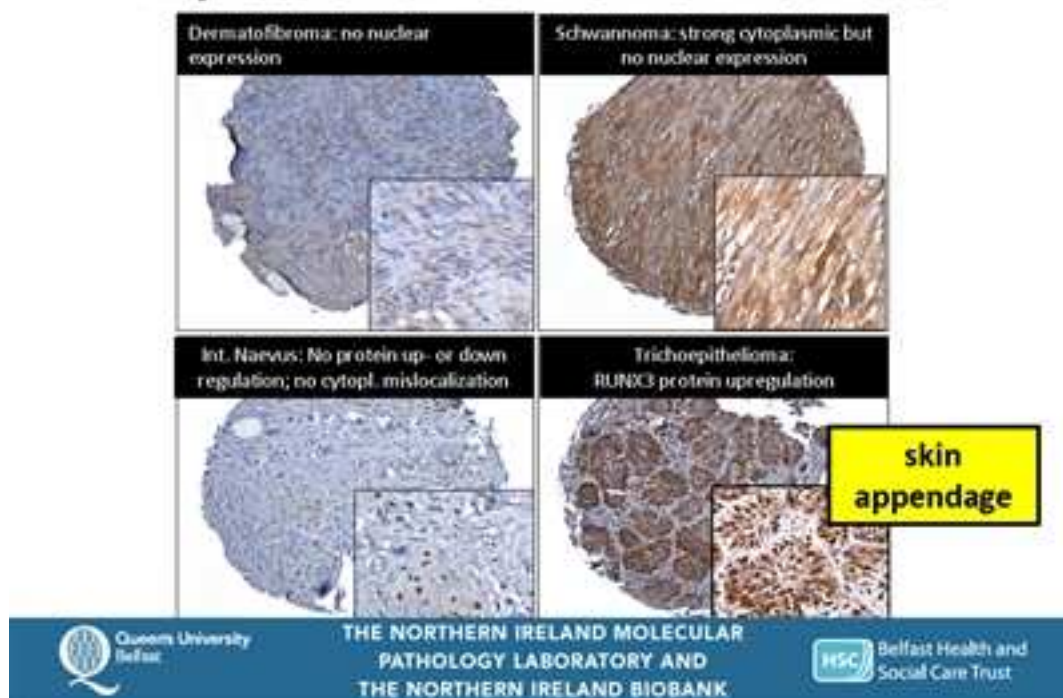
THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Expression of RUNX3 in skin tumors



Oncogene

Oncogene (2006) 1-4
© 2006 Nature Publishing Group. All rights reserved. 0950-0607/06 \$30.00
www.nature.com/onc

SHORT COMMUNICATION

RUNX3 protein is overexpressed in human basal cell carcinomas

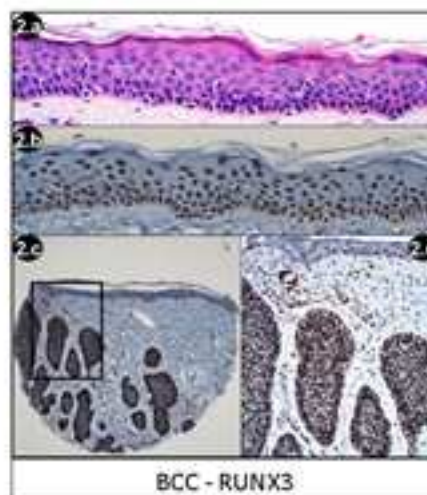
M Salto-Tellez^{1,2}, BK Peh^{1,2}, K Ito^{1,2}, SH Tan¹, PY Chong^{1,2}, HC Ham¹, K Tada², WY Ong¹,
R Soong^{1,2}, DC Voon¹ and Y Ito^{2,4}



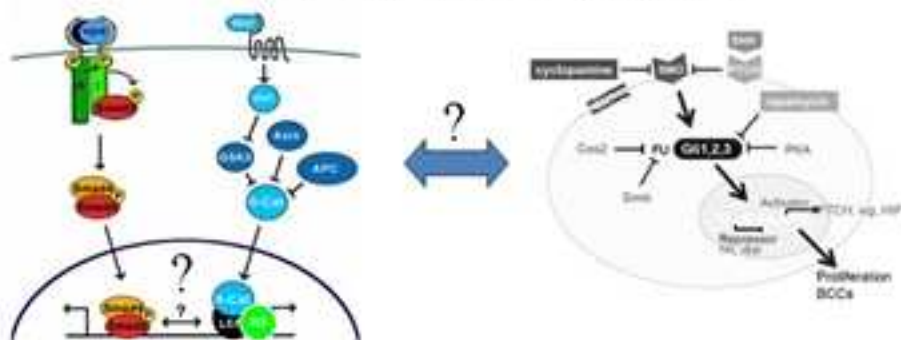
Results



Figure 3 Western blot analysis of RUNX3 expression. Whole-cell extracts from COS-7 cells expressing exogenous RUNX3; normal skin cell line CRL-7701 (American Type Culture Collection (ATCC), Manassas, VA, USA); BCC-derived cell line CRL-7702 (ATCC); and gastric cancer line SNU-5 (ATCC), which co-expresses endogenous RUNX3. Western blot was performed using RUNX3-specific monoclonal antibody B3-SG4, as described by Ito *et al.* (2007).



RUNX3? TGFβ / WNT / SHH



Peh BK (Salto-Tellez). RUNX3 and the Sonic Hedgehog Pathway (2013) – Under review



Tissue Microarray sets

MORPHO-MOLECULAR CARCINOGENESIS ARRAY

CRC	RCC	Lymphoma
Gastric Ca	Ovarian Ca	General
HCC	DermPath (skin multiple)	Lung (small)
Breast Ca	BCC	Lung (big)
Ovarian Ca	NPC	Prostate
Sarcoma (translocation-related)	Medulloblastoma	
SarcPath (sarcoma multiple)	Gastritis-IM-Dysplasia-GC	



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



British Journal of Cancer (2011) 105, 658–665
© 2011 Cancer Research UK. All rights reserved 0007-1226/11
www.bjpcancer.com

Sequential expression of putative stem cell markers in gastric carcinogenesis

T Wang¹, CW Ong¹, J Shi², S Srivastava¹, B Yan¹, CL Cheng², WP Yong¹, SL Chan¹, KG Yeoh¹, B Iacopetta³,
M Salto-Talila^{4,5,6} and the Singapore Gastric Cancer Consortium

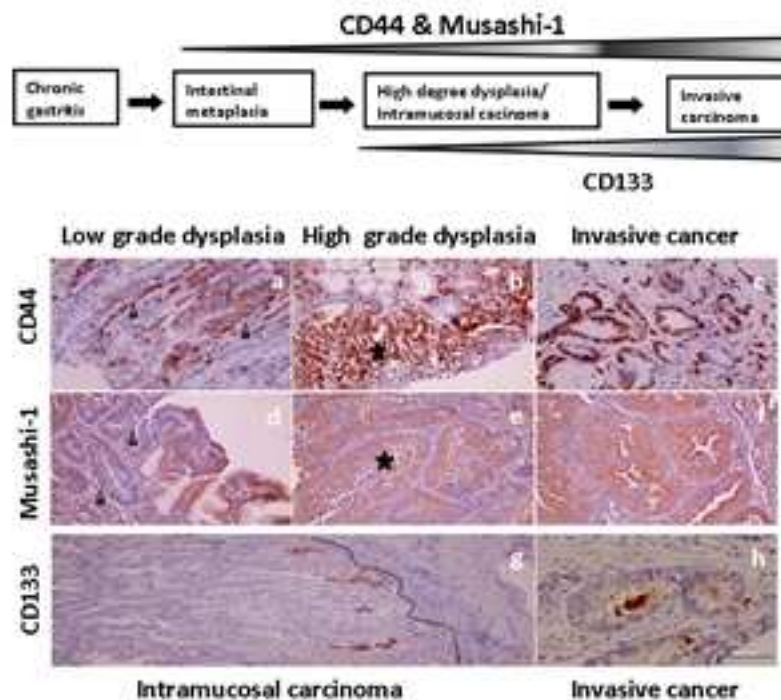
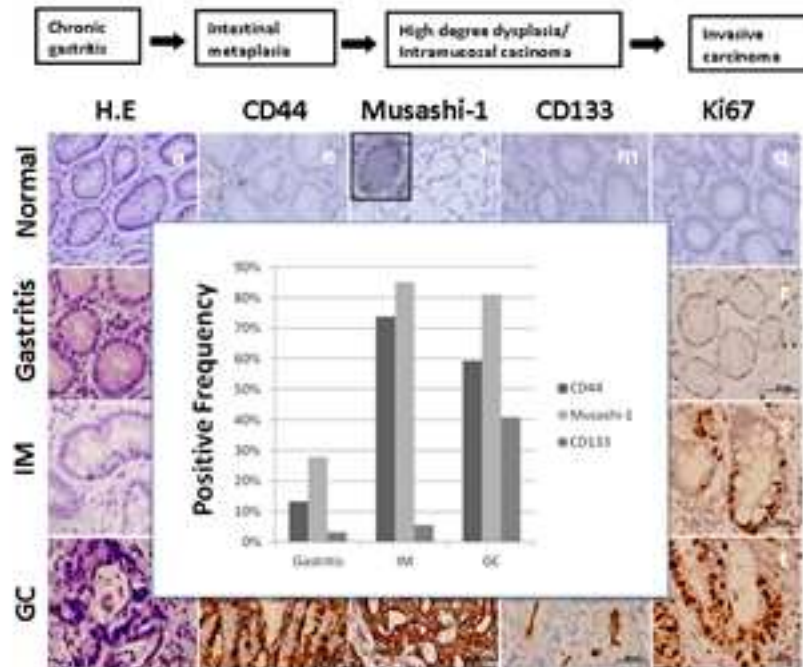


Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

NCBI Resources How To

PubMed
U.S. National Library of Medicine
National Institutes of Health

Published | Advanced

Display Settings: Abstract Send to:

Oct 2012 Dec 01/12:1679-85. doi: 10.1136/gutjnl-2011-301193. Epub 2011 Dec 23.

Spasmolytic polypeptide-expressing metaplasia (SPEM) in the gastric oxyntic mucosa does not arise from Lgr5-expressing cells.

Nehls KT, O'Neill RL, Coffey RJ, Finke PE, Barker N, Goldstein JS.
Nashville VA Medical Center and the Department of Surgery, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-2733, USA.

Abstract

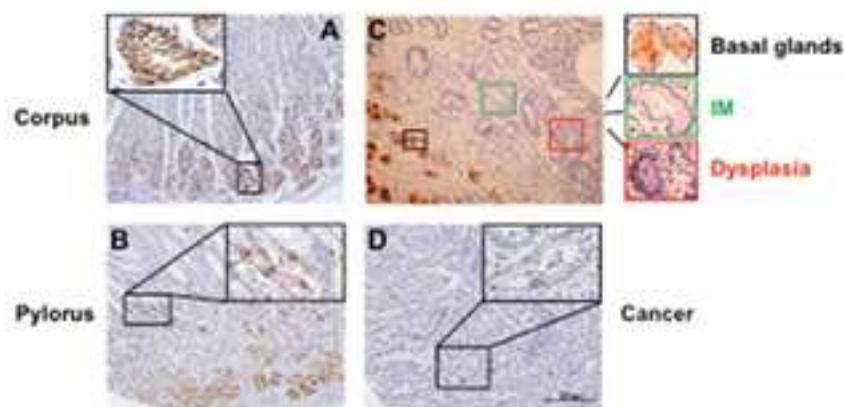
OBJECTIVE: Metaplastic lineages in the oxyntic mucosa of the stomach are critical preneoplastic precursors of gastric cancer. Recent studies have demonstrated that spasmolytic polypeptide-expressing metaplasia (SPEM) in the mouse oxyntic mucosa arises from transdifferentiation of mature gastric chief cells. Other investigations of intestinal progenitor cells have shown that cells demonstrating transcriptional activity for leucine-rich repeat containing G-protein-coupled receptor 5 (Lgr5) in the intestine, colon and gastric antrum function as adult stem cells. We have now investigated whether cells demonstrating Lgr5 transcriptional activity in the oxyntic mucosa of mice might be responsible for development of metaplasia.

DESIGN: Lgr5-EGFP-IRES-Cre(ERT2/+);Rosa26R mice were used to examine the distribution of Lgr5 transcriptionally active cells in the normal oxyntic mucosa as well as after treatment with DMP-777 or L-635 to induce acute SPEM. Lineage mapping was performed to determine if Lgr5-expressing cells gave rise to SPEM.

RESULTS: Cells expressing transcriptional activity for Lgr5 in the oxyntic mucosa were present as scattered rare cells only along the lesser curvature of the stomach. These cells also stained for markers of chief cells (intrinsic factor and pepsinogen) but never showed any staining for proliferative markers (Ki-67). In Lgr5-EGFP-IRES-Cre(ERT2/+);Rosa26R mice induced with tamoxifen, treatment with either DMP-777 or L-635 to induce acute oxyntic atrophy caused induction of SPEM, but no lineage mapping into SPEM from Lgr5-expressing cells was observed.

CONCLUSION: The results indicate that, while chief cells with Lgr5 transcriptional activity are present along the lesser curvature of the gastric oxyntic mucosa, they are not responsible for production of metaplasia.

Comment in
Lgr5 expression is absent in human premalignant lesions of the stomach. [Gut. 2012]



LETTER

Lgr5 expression is absent in human premalignant lesions of the stomach

Gut 2012; ■ 1. doi:10.1136/gutjnl-2012-302372

Tingling Wong,¹ Khay Guan Yeoh,¹
Manuel Salto-Talero^{2,3}

¹Cancer Science Institute Singapore, National University Health System & National University of Singapore, Singapore; ²Centre for Cancer Research and Cell Biology, Queen's University, Belfast, UK;



THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

1. Prospective design	Define clear objectives
	Design experiment to answer the specific research question
	Identify and organise any clinical data required
	Consult biostatistician and ensure that study is adequately powered
	Write a protocol for the study, including scoring, staining and data analysis methods



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

Tissue Microarray sets

CLINICAL PROGNOSTIC / PREDICTIVE ARRAY

CRC	RCC	Lymphoma
Gastric Ca	Ovarian Ca	General
HCC	DermPath (skin multiple)	Lung (small)
Breast Ca	BCC	Lung (big)
Ovarian Ca	NPC	Prostate
Sarcoma (translocation-related)	Medulloblastoma	GIST
SarcPath (sarcoma multiple)	Gastritis-IM-Dysplasia-GC	



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

TISSUE MICROARRAY	
CANCER TYPE:	Colorectal Cancer
NUMBER OF TUMOR SAMPLES	610 (1990-1999)
NUMBER OF PAIRED NORMAL SAMPLES	610 (1990-1999)
NUMBER OF PATHOLOGICAL DATA COLLECTED	64
NUMBER OF CLINICAL DATA COLLECTED	50
FOLLOW-UP	Max of 20.5 years
BIOMARKERS ANALYSED TO DATE	bmp4, CD44, CD133, CEP68, Ck7, Ck20, Cox2, jag1, Ki67, Lgr5, NHE-1, Oct4, p27, p53, PTEN, Sox2, Stathmin, Sphingosine kinase 1



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

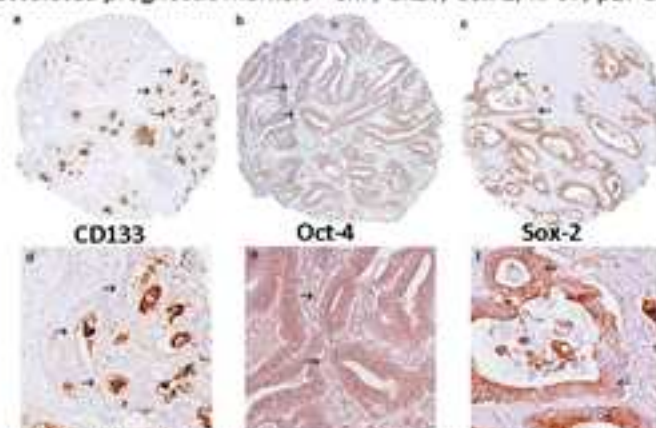
CD133 expression predicts for non-response to chemotherapy in CRC

We examined both the prognostic and predictive significance of putative cancer stem cell markers in colorectal cancer.

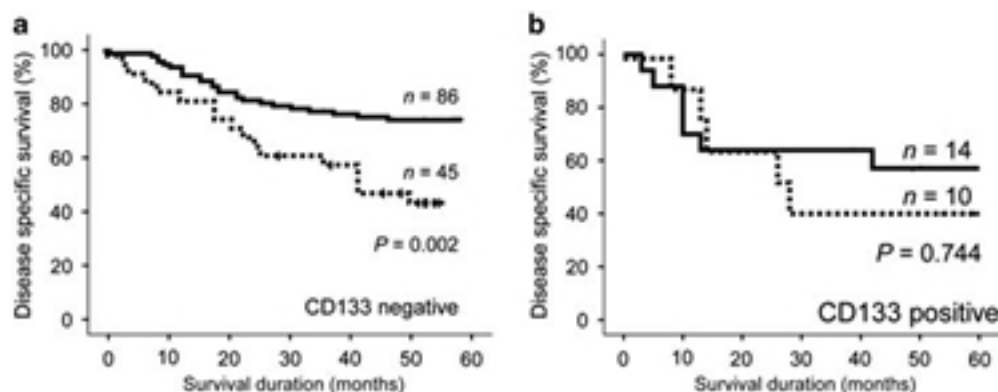
Immunohistochemistry for:

Three candidate cancer stem cell markers - CD133, Oct-4 and Sox-2 and

Six other postulated prognostic markers - CK7, CK20, Cox-2, Ki-67, p27 and p53



Ong CW, (Salto-Tellez M). *Mod Pathol.* 2010 Mar;23(3):450-7

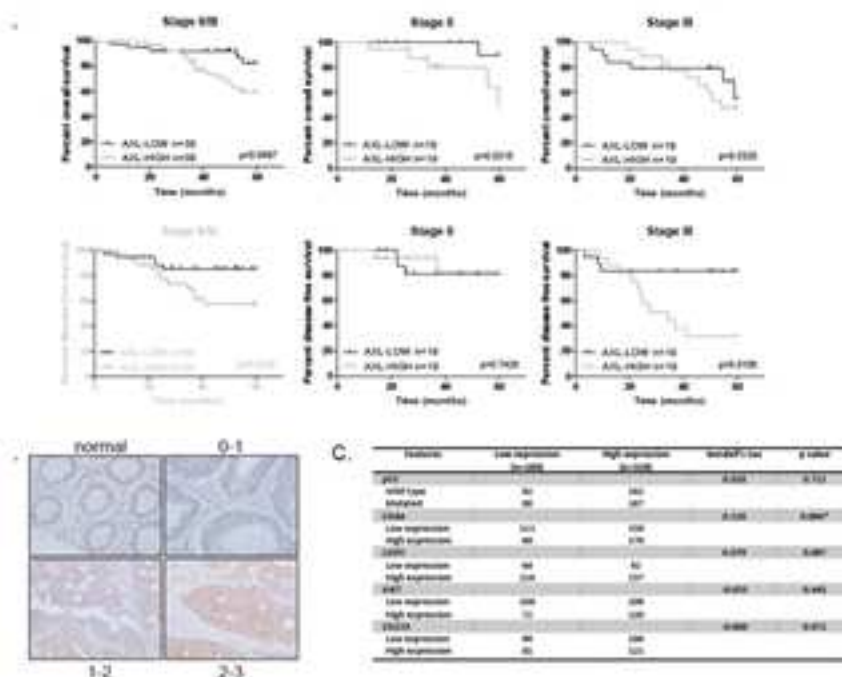


Kaplan-Meier survival analysis of stage III colorectal cancer patients according to negative (a) or positive (b) expression of CD133, Oct-4 and Sox-2, respectively.

Broken lines represent patients treated by surgery alone, while continuous lines represent patients treated with adjuvant chemotherapy in addition to surgery.

An apparent survival benefit from chemotherapy is observed for those patients with no CD133 expression, but not for those with positive CD133 expression.

Ong CW, (Salto-Tellez M). Mod Pathol. 2010 Mar;23(3):450-7



AXL is a key regulator of inherent and chemotherapy-induced migration/invasion and a poor prognostic marker in early stage colon cancer. Cancer Research 2013, in press



Education and Culture DG

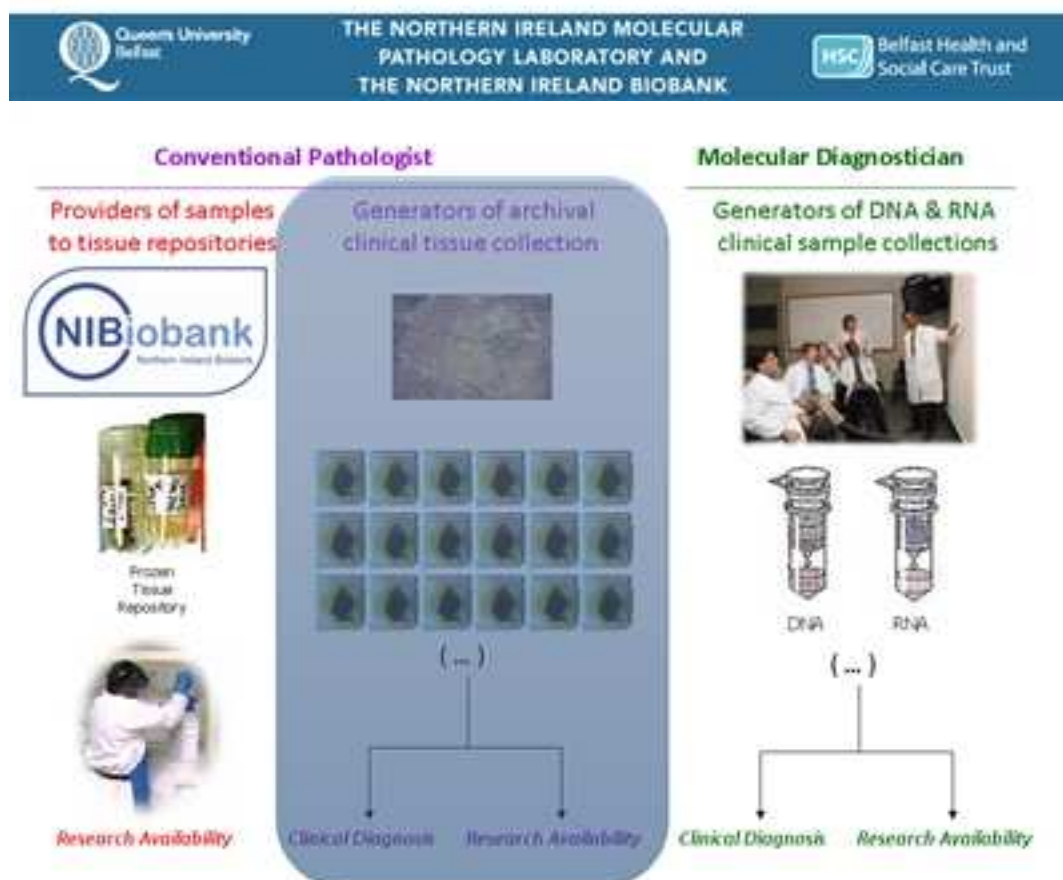
Lifelong Learning Programme

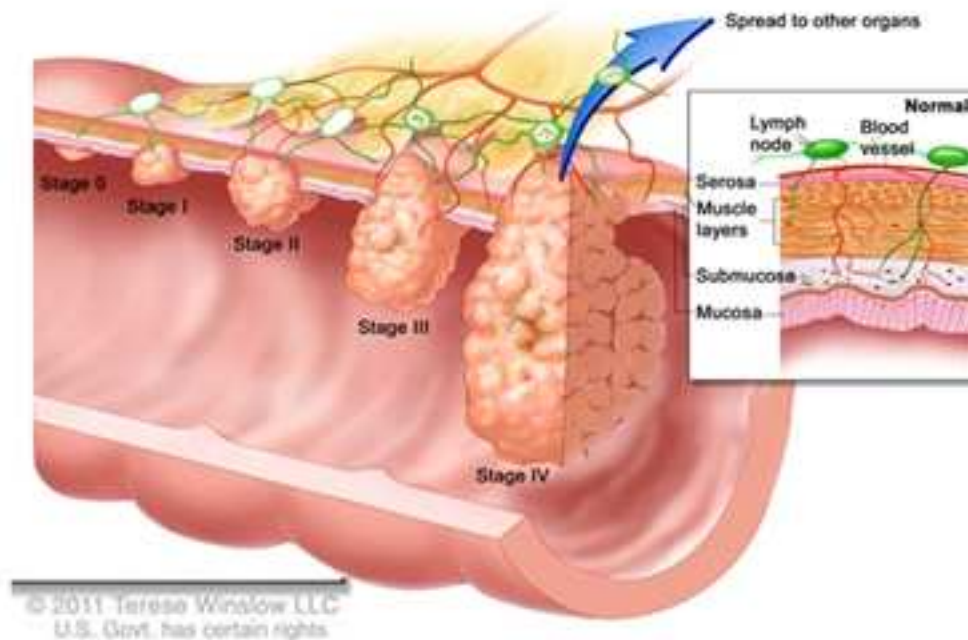
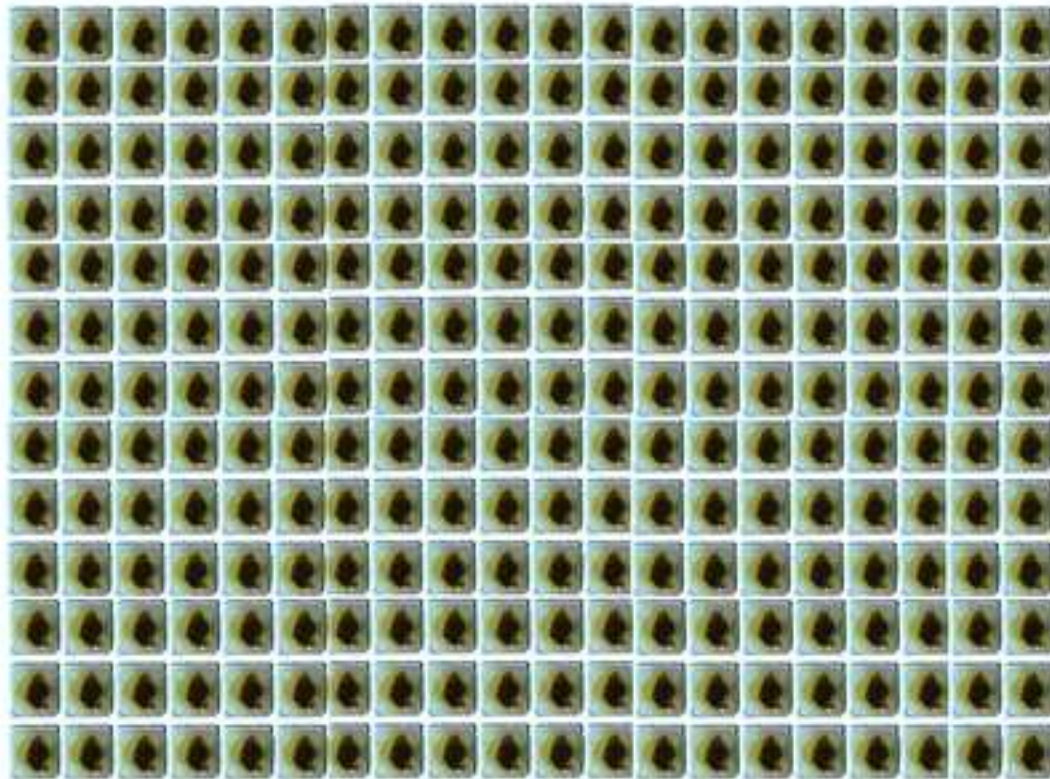
EACEA

Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

2. Collect tissue	Ensure review of cases by expert histopathologist to choose best block
	For prospective collections, write protocol to ensure standardised tissue handling
	Compare profiles of collected cases with overall study population to assess potential bias introduced by missing cases





[illegible]

Templates and data accumulation

Type	Defined = FDF	Data on system	Collector
breast	1 Template Defined	1 Data on Film	1 Garrett Davis and David Boyle
MAN	1 Template Defined	1 Data on Film	1 Michael Moran
Ovarian	1 Template (redefined)	1 Data on Film	1 Originally Judith Carter
Colonial	1 Template Defined	1 Data in Collection	1 Clive Norrie and Maurice Hughes
Colonial	1 Template Defined	1 Data in Collection	1 Richard Lunnington
Prostate	1 Template in preparation	1 New Protocol to begin collection	1 Tarell Jain
Colonial	1 Template in preparation	1 Applications gone to studies	1 Donald Suggs





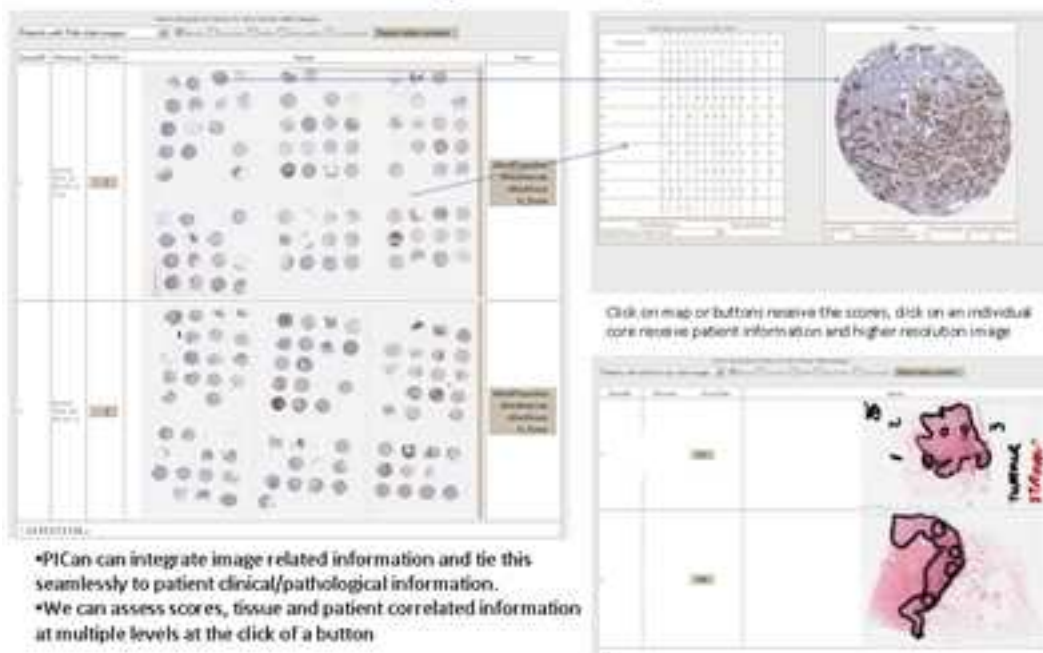
Education and Culture DG

Lifelong Learning Programme

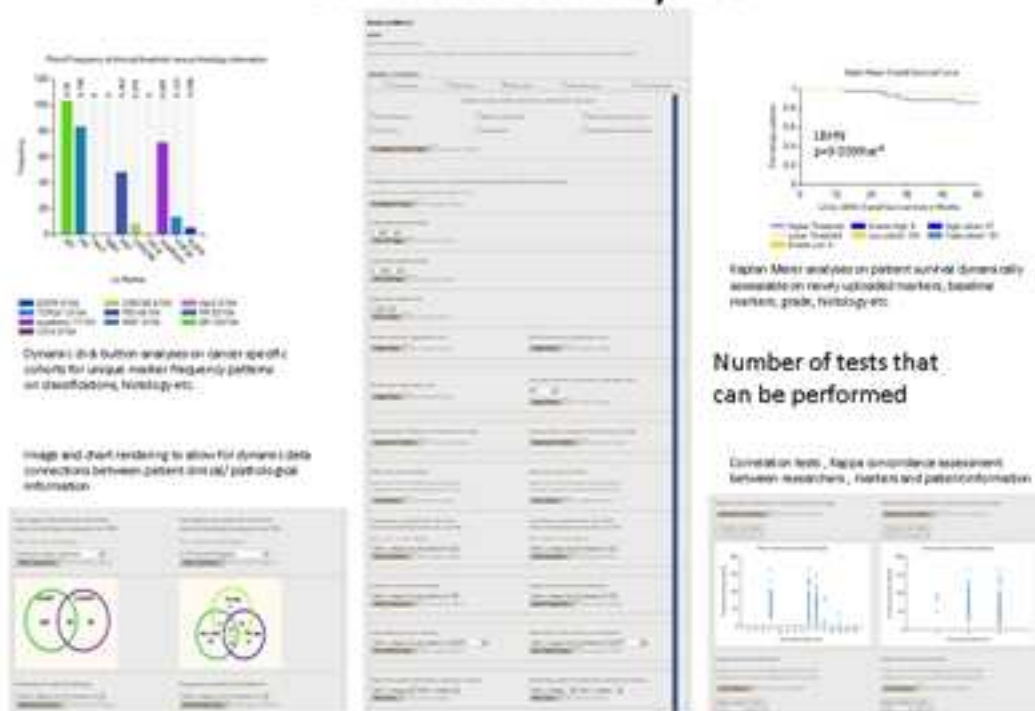
EACEA

Education, Audiovisual & Culture
Executive Agency

Image analysis

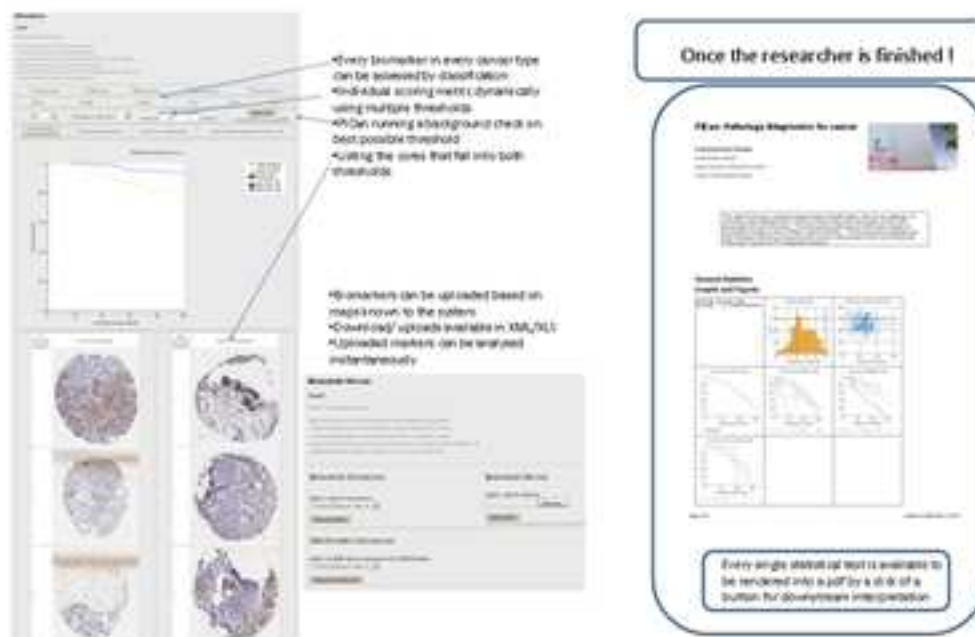


Statistical analyses





Novel Biomarker assessment



Histopathology. 2013 May;62(6):827-39.

3. Mark slides and blocks

Ensure H&E sections and blocks are reviewed and areas of interest are marked by expert Pathologist

Choose core number, core size and sampling sites in accordance with the underlying research question and heterogeneity of tissue

Choose appropriate controls for inclusion in each TMA block

Consider additional cores or sections for DNA/RNA extraction for complementary studies



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

Core size

The most common core size is 0.6-mm diameter, although larger cores of 1-, 1.5- or 2-mm diameter may be more appropriate if, for example, the study is of diffusely infiltrative tumours/tumour edges/rare events/tumour-associated stroma, if the tumour is stroma-rich, or if the tumour contains abundant adipose tissue (which increases the risk of core loss). The benefit of larger cores needs to be balanced against the extra time required to examine a greater area of tissue, a reduction in the numbers of cores per TMA, and the possible effects of larger cores on the physical integrity of both the donor and recipient blocks. Some studies claim that larger core sizes provide little benefit, and it is preferable to have more multiple cores of standard size.²¹ Thus, three cores of 0.6-mm diameter will have a similar area to one core of 1-mm diameter (0.85 mm² versus 0.78 mm², respectively), but will potentially yield more information about the tumour as they are likely to represent different areas.



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

Histopathology. 2013 May;62(6):827-39.

Core number and sampling

The number of cores to be taken from a tissue block will be determined by the research question and the degree of heterogeneity within the tumour being studied. Heterogeneity may be intrinsic (arising from subclonal evolution in tumours or due to variation in tumour differentiation or tumour microenvironment) or extrinsic (arising from technical sources such as variation in fixation or ischaemia prior to fixation).²²⁻²⁶ Ideally, each TMA would be tailor-made in accordance with the requirements of each experiment. However, the reality is that TMAs need to be constructed with some degree of 'future-proofing' to allow as-yet unconsidered studies to be undertaken. While early studies suggested that a single core may be sufficient,²⁷ several subsequent studies have suggested that between two and four cores of 0.6-mm diameter will give adequate representation. Commonly, three cores are taken¹⁸ and this is probably an acceptable rule of thumb, although more heterogeneous tissues will require a greater number of cores.²⁸ Sampling may be 'random' or, if regional variation is expected, cores may be targeted to ensure that all regions are included in the experiment. Virtual sampling on digital images of WTS can be performed to estimate of the number of cores required for adequate representation.²⁹



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

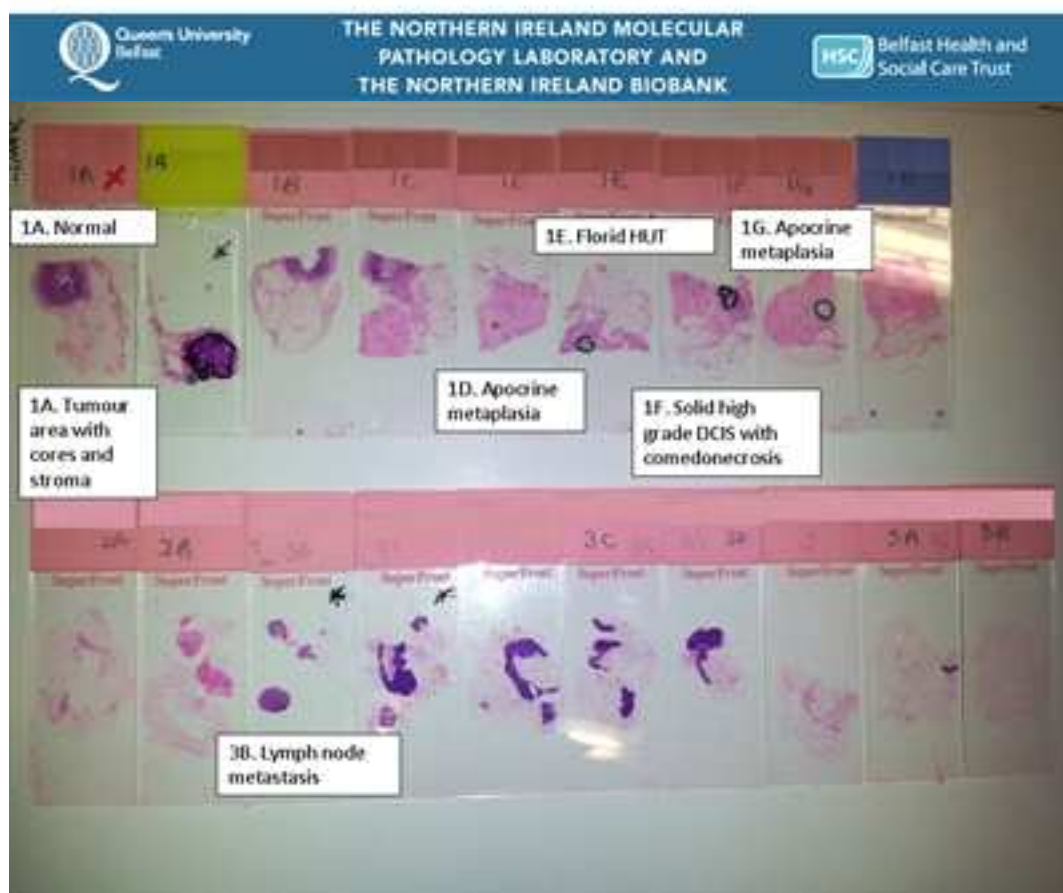


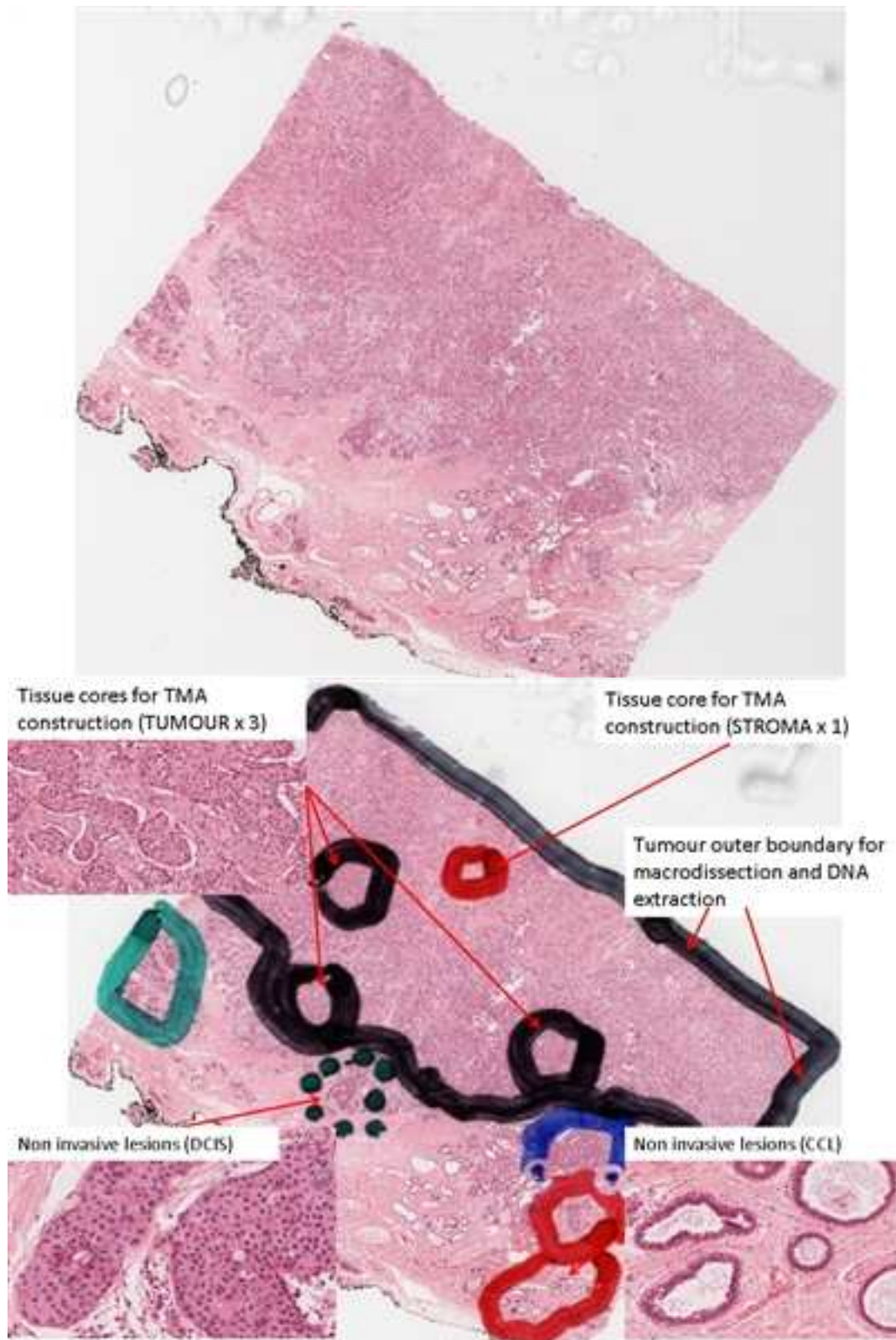
WHEN PREPARING A "GENERAL" ARRAY:

Annotate / array as many cases as possible
Collect as much clinico-pathological information as possible

Normal (x3),
Invasive (x3),
Stroma (x1),
Tumour associated inflammation (x1),
preneoplastic lesions,
lymph node metastases

10 tissue arrays per 100 cases







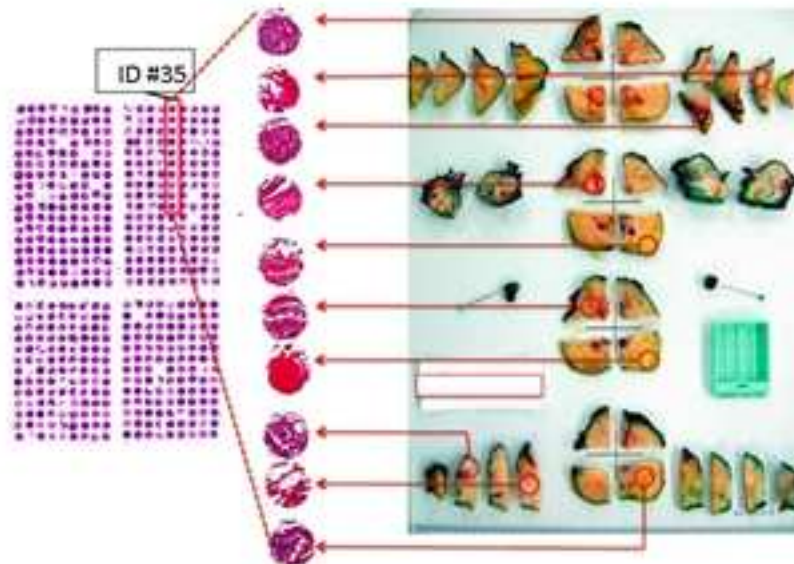
Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

HETEROGENEITY



Chapter 9 - Simon & Mirlacher
B. Jordan (ed.), *Microarrays in Diagnostics and
Biomarker Development*
Springer-Verlag Berlin Heidelberg 2012.

Histopathology. 2013 May;62(6):827-39.

Normal tissues and positive controls

Inclusion of normal tissue in the TMA will depend upon the research question. Thus, biomarker expression in normal and tumour tissue may be the best approach for discovery of tumour specific targets but, if the study is of prognostic or predictive markers, biomarker expression in normal tissue is irrelevant.

Inclusion of positive controls in each block is essential in order to confirm technical reliability of the immunostaining and to allow normalization of data (see below) between different TMA sections. A variety of positive controls may be used, including tissues known to express the biomarker, cell lines expressing the biomarker (either naturally or by forced expression) and sepharose beads to which the biomarker has been physically conjugated. Selecting appropriate controls at the time of TMA construction for future unknown experiments is impossible. Potential solutions include adding cores from several different tissues in the TMA block (in anticipation that some may naturally express the biomarker) or adding a section from a known control tissue onto the glass slide adjacent to the TMA section.



THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



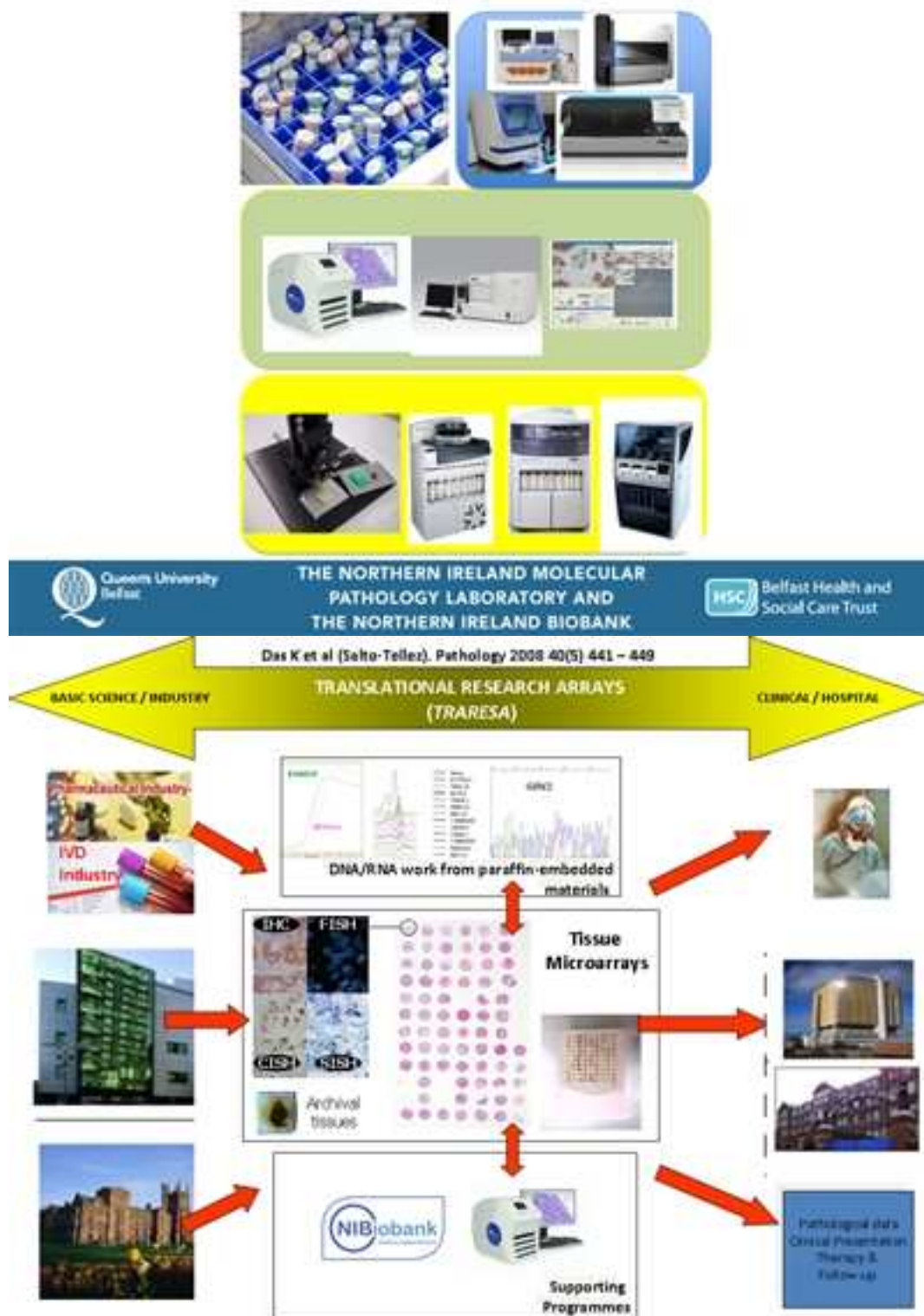
Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

MOLECULAR PATHOLOGY PROGRAMME IN BELFAST – THE LABORATORY





Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

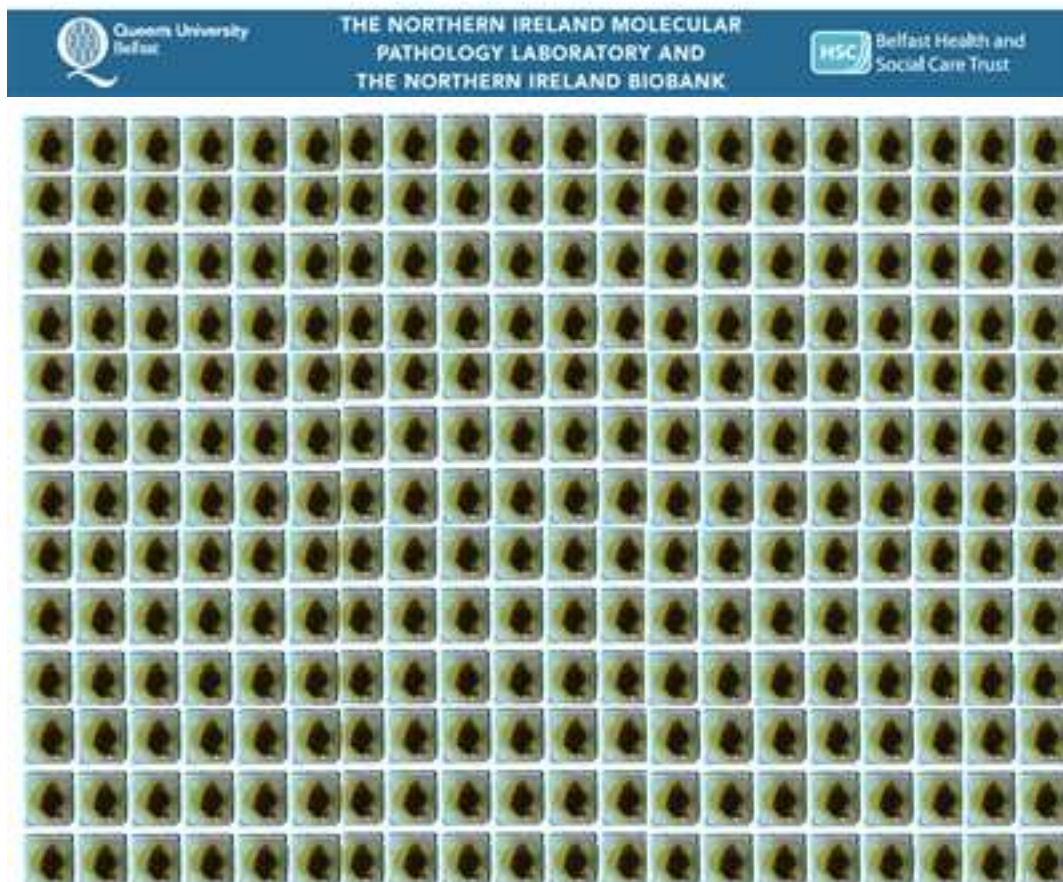
4. Construct TMA map

Design asymmetric grid layout for the TMA

Consider 'marker' cores at specific co-ordinates. For high density arrays, consider grid design with sub-arrays

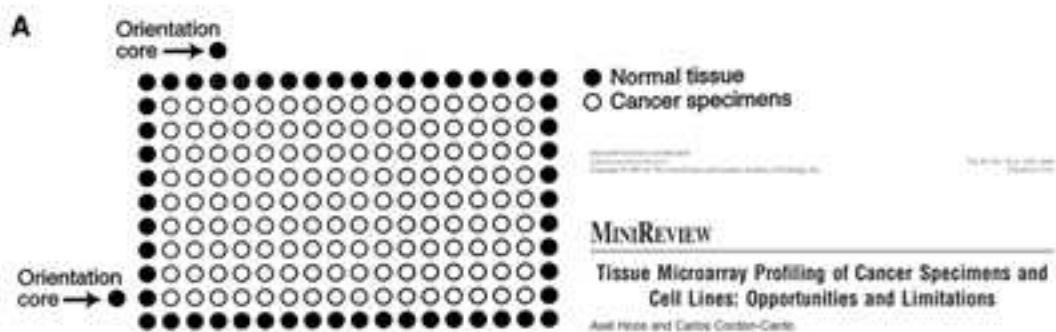
Disperse cores randomly in the grid and, if taking multiple cores per case, allocate each core from a case to a different recipient block

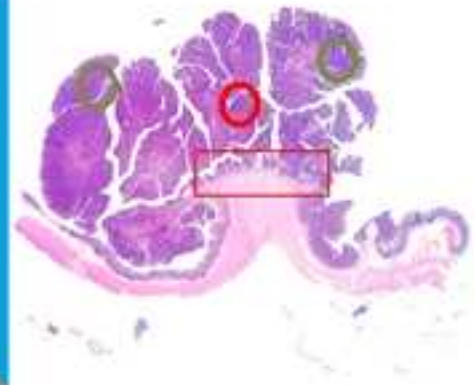
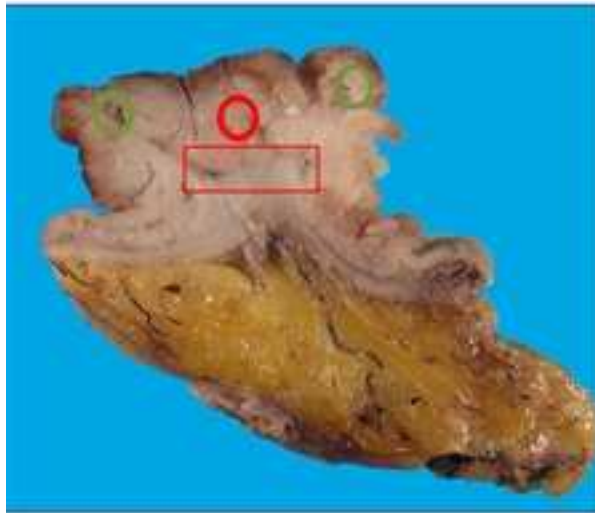
If sampling non-randomly from specific regions (such as tumour edge), consider 'region-specific' TMAs





	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
1	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
2	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
3	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
4	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
5	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150
6	BT 101	BT 102	BT 103	BT 104	BT 105	BT 106	BT 107	BT 108	BT 109	BT 110	BT 111	BT 112	BT 113	BT 114	BT 115	BT 116	BT 117	BT 118	BT 119	BT 120	BT 121	BT 122	BT 123	BT 124	BT 125	BT 126	BT 127	BT 128	BT 129	BT 130	BT 131	BT 132	BT 133	BT 134	BT 135	BT 136	BT 137	BT 138	BT 139	BT 140	BT 141	BT 142	BT 143	BT 144	BT 145	BT 146	BT 147	BT 148	BT 149	BT 150





Histopathology. 2013 May;62(6):827-39.

5. Construct TMA

Ensure recipient block is of sufficient quality

Ensure sufficient distance from edge of block to the nearest core

Consider taking out paraffin cores from the recipient block to decrease pressure

Consider adding special matrix for high density arrays

Ensure H&E section of each TMA block is examined by an expert histopathologist



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

TISSUE MICROARRAY CONSTRUCTION DEPENDS ON:

THE TYPE OF TISSUE ARRAY

THE TYPE OF TISSUE ARRAYER

THE TYPE OF STUDY – SCIENTIFIC QUESTION



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

TYPES OF TISSUE ARRAYS

Human full resection specimens - FFPE

1. Recipient block technology
2. Cutting-edge matrix assembly (CEMA)
3. Hypodermic needle technology
4. Resin tissue microarray technology

Human non-FFPE

1. Frozen tissue samples

Human non- full resections

1. Core needle biopsies

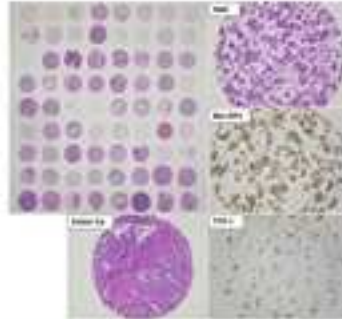
Experimental tissues

e.g. xenografts

Cells in suspension

e.g. cell lines

Chapter 9 - Simon & Mirlacher
B. Jordan (ed.), *Microarrays in Diagnostics and
Biomarker Development*
Springer-Verlag Berlin Heidelberg 2012



Cell block - TMA

Howat W J et al. Journal of Clinical Microbiology 2005; 43: 1189-1197

JCL JOURNAL OF CLINICAL MICROBIOLOGY

RESEARCH

Open Access

Automated ERCC1 immunohistochemistry on hybrid cytology/tissue microarray of malignant effusions: evaluation of antibodies 8F1 and D-10

Howat W J et al. Journal of Clinical Microbiology 2005; 43: 1189-1197



1A

20-tissue resin TMA

Howat W J et al.
J Histochem Cytochem
2005; 53: 1189-1197



Cryo-TMA



Xenograft-TMA



TYPES OF TISSUE ARRAYERS

No Tissue Arrayer



Manual Tissue Arrayer

Semi-automated Tissue Arrayer

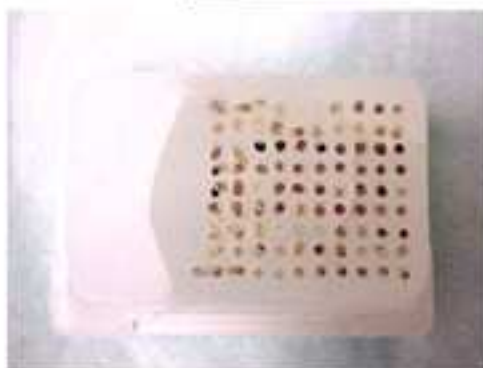
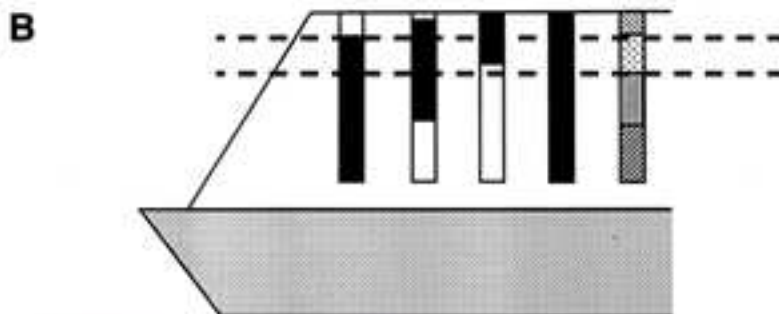


Fully Automated Tissue Arrayer



Histopathology. 2013 May;62(6):827-39.

6. Cut sections	Ensure TMA sections are cut by appropriately trained person
	Cut sections in batches to minimise tissue loss
	Keep excess tissue as spare sections for optimisation experiments.
	Store sections if not to be used immediately in conditions which will help protect against tissue oxidation



Downloaded from ascelibrary.org by
University of Illinois at Chicago on 07/10/13. Copyright ASCE. For personal use only; all rights reserved.

DOI: 10.1061/(ASCE)1080-4022(2013)108:4(4022)

MiniReview

Tissue Microarray Profiling of Cancer Specimens and Cell Lines: Opportunities and Limitations

Andrzej Huczy and Carlos Cordon-Cardo



Education and Culture DG

Lifelong Learning Programme



Education, Audiovisual & Culture
Executive Agency

Histopathology. 2013 May;62(6):827-39.

CUTTING AND STORAGE OF THICKED MICROARRAY SECTIONS

Cutting sections from a TMA is technically more challenging than cutting WTS and is prone to specific artefacts, such as variation in the thickness across the section and gross distortion of the section (resulting in loss of orientation of the cores). Special training in cutting TMA sections will reduce tissue loss due to such artefacts, and novel techniques, such as the 'tape-transfer' method, may also reduce sectioning artefacts.³⁴ Sections should be cut in batches to avoid tissue loss which occurs when refacing the block to cut new sections. It may be useful to keep any 'waste' section from the TMA block (e.g. those that are not full face) for optimising the downstream tests.

Once sections have been cut, if not used immediately they should be stored in order to reduce loss of biomarker due to tissue oxidation. A variety of protocols exist which are designed to maintain biomarker antigenicity. These include storing sections at 4, -20 and -80°C; dipping sections in wax; cutting an empty paraffin block and placing this 'empty' section on top of the TMA section; and dehydrating the sections in a vacuum.³⁵ Data on the comparative efficacy of these methods are not available, and our collective experience is that data derived from sections more than 2 months old should be treated with caution.



THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



The SALL4 Story



SALL4 is the human homologue of the *Drosophila* spalt homeotic gene

SALL4 encodes a C2H2 zinc finger transcription factor.

It is one of the key factors for maintenance of pluripotency and self renewal of embryonic stem cells.

In recent years, SALL4 has emerged as a novel oncogene, first reported in leukemia (AML)

De Celis JF, Barrio R. *UDB* 2009;53:1385-98.

Elling U, et al. *PNAS* 2006;103:16319-24.

Oikawa T, et al. *Gastroenterology* 2009;136:1000-11.

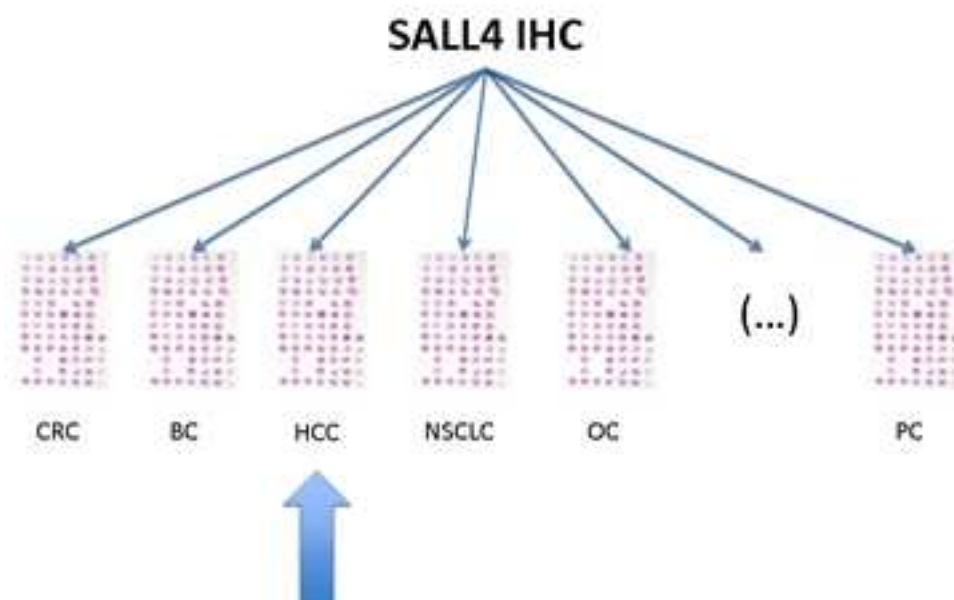
Ma Y, et al. *Blood* 2006;108:2726-35.



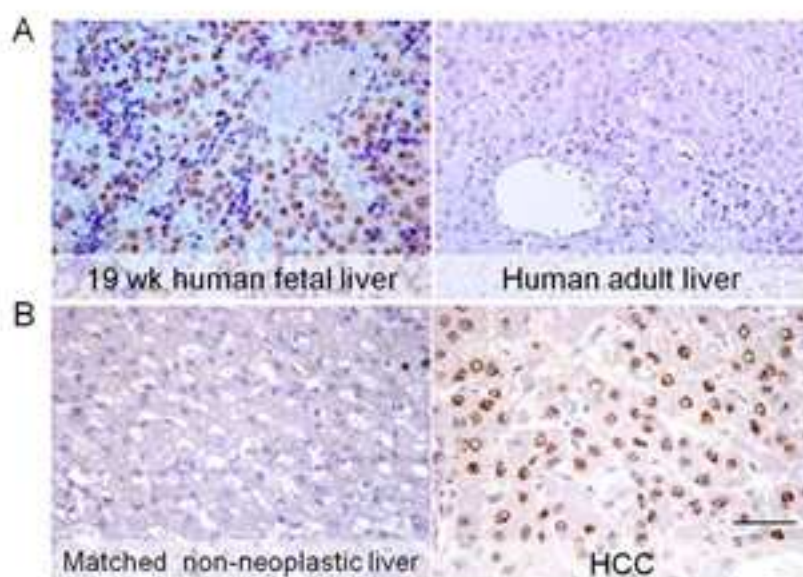
THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



SALL4 & HCC



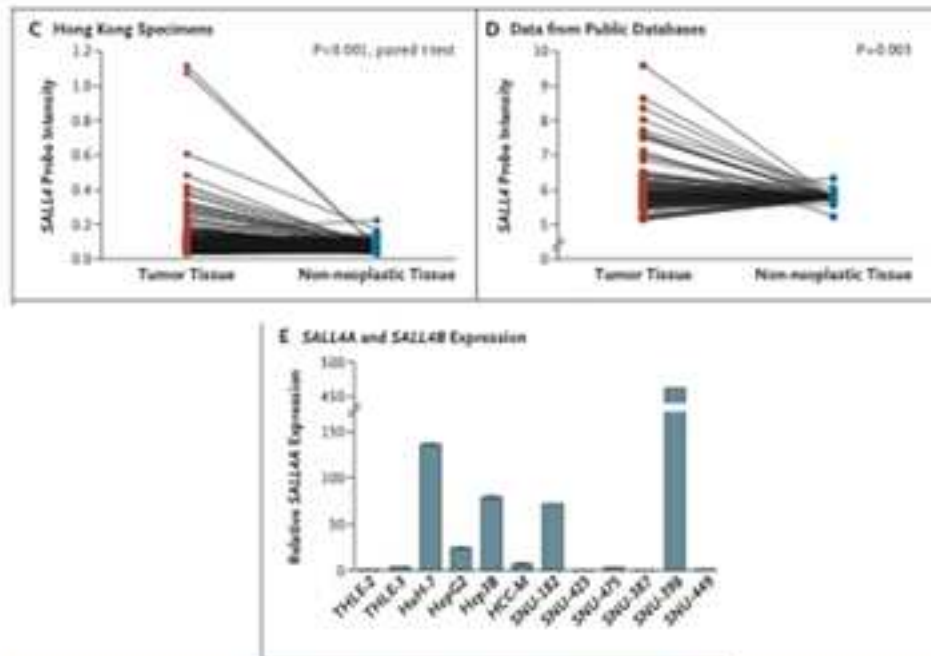


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

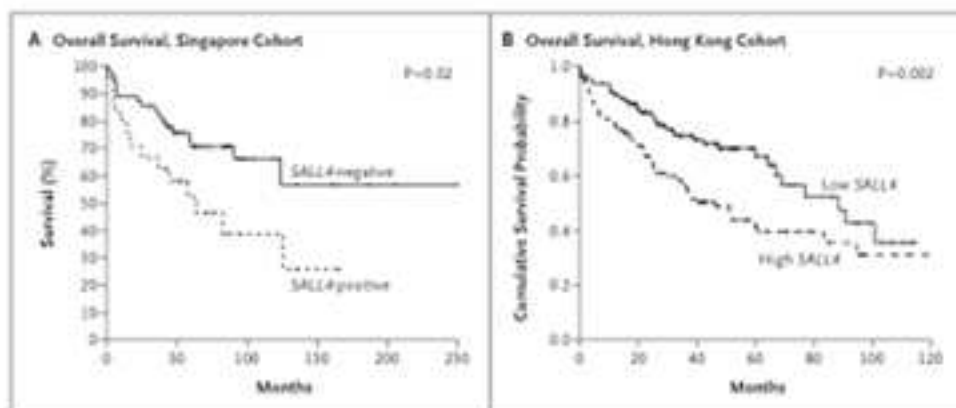


Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust



Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency

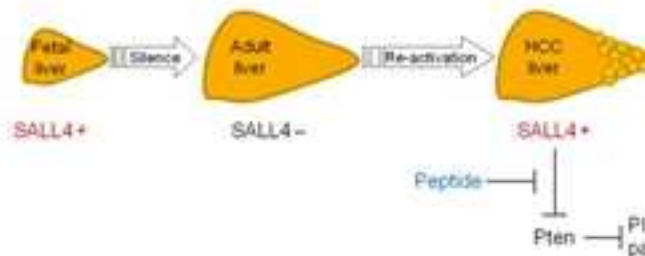


Figure S2. SALL4 is a novel oncofetal protein in HCC. In healthy humans, SALL4 is expressed in fetal liver but silenced in mature adult liver. In a subgroup of HCC livers, SALL4 is re-activated and plays a functional role in hepatocarcinogenesis by silencing the tumor suppressor PTEN through the recruitment of the NuRD complex. A therapeutic peptide can be used to block the interaction between SALL4 and the NuRD complex, thereby activating *PTEN* transcription. Upregulation of PTEN expression leads to downregulation of pAKT level and silencing of the PI3K/AKT survival signaling, resulting in decreased HCC cell viability and tumorigenicity. We propose SALL4 to be a novel oncofetal protein that can be specifically targeted for treatment of a subgroup of aggressive HCCs with SALL4 expression.



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

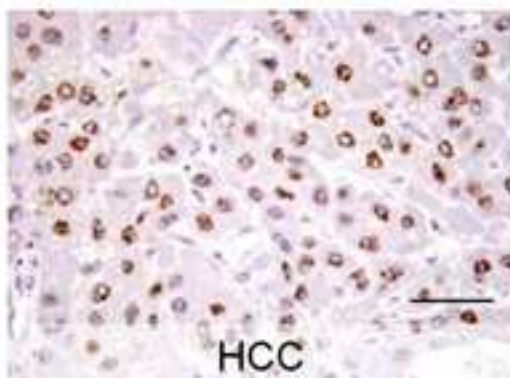
Kai Jia Yang, B.Sc., Qiong Gao, M.D., Ph.D., Joline S.J. Lim, M.B., B.S.,
Benedict Tan, M.B., B.S., Harry Yang, Ph.D., Tudor Dimitrov, Ph.D.,
Amita Kawasaki, M.D., Ph.D., Chee Wei Ong, M.Sc., Kwong Fai Wong, Ph.D.,
Sanghoon Lee, Ph.D., Sharada Ravikumar, M.D., Ph.D., Supriya Srivastava, M.D.,
Xi Tian, B.S., Renise T. Poon, M.B., B.S., Ph.D., Sheung Tat Fan, M.D., D.Sc.,
John M. Luk, O.Med.Sc., Yock Young Dan, M.B., B.S., Ph.D.,
Manuel Salto-Tellez, M.D., Li Chai, M.D., and Daniel C. Tenen, M.D.



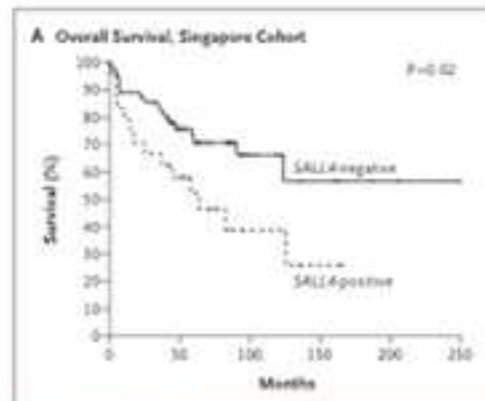
THE NEW ENGLAND JOURNAL OF MEDICINE

www.nejm.org | ISSN 0028-2718 | Volume 369, Number 12, June 13, 2013

**Oncofetal Gene SALL4 in Aggressive
Hepatocellular Carcinoma**



HCC



Queen's University
Belfast

THE NORTHERN IRELAND MOLECULAR
PATHOLOGY LABORATORY AND
THE NORTHERN IRELAND BIOBANK



Belfast Health and
Social Care Trust

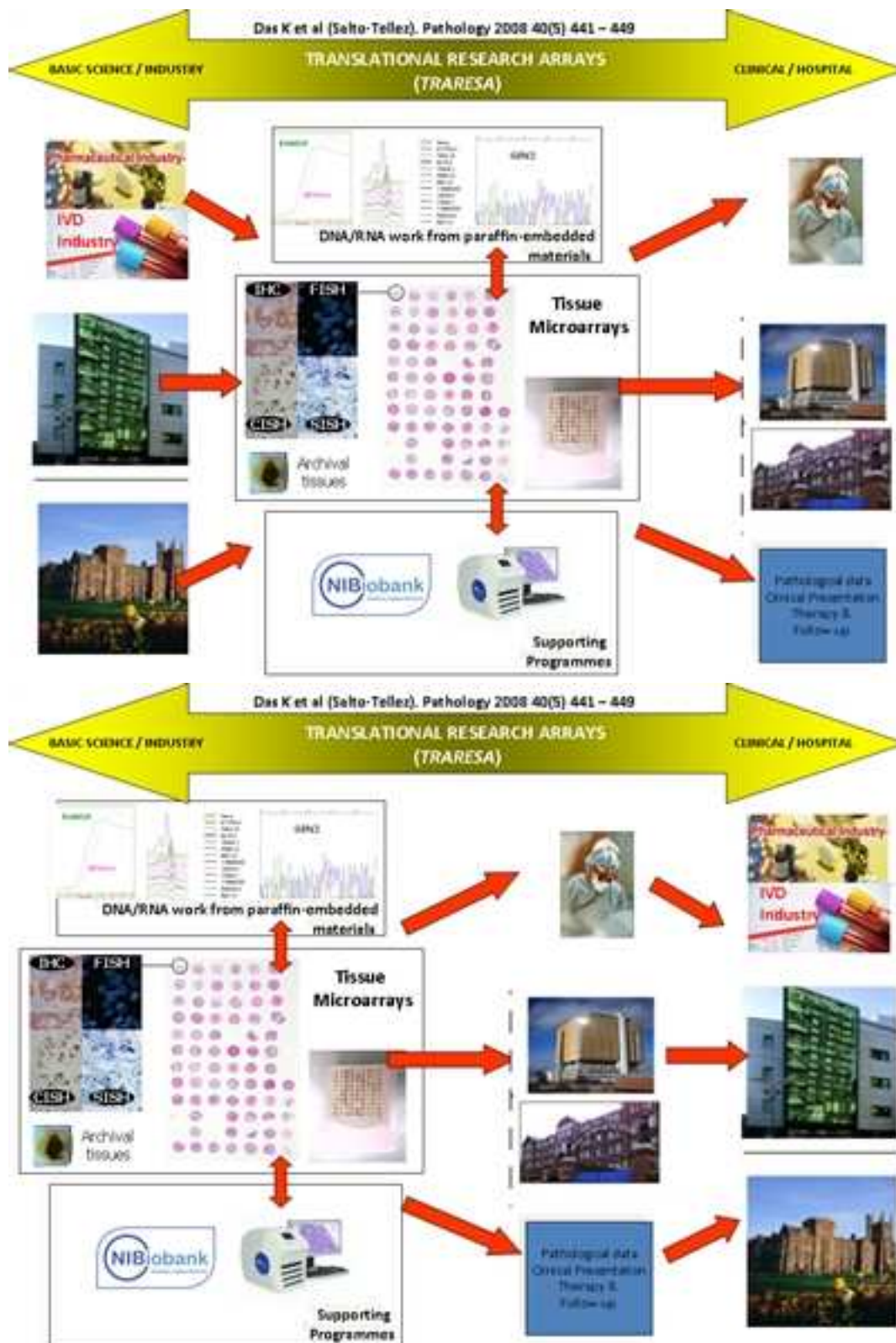


Education and Culture DG

Lifelong Learning Programme

EACEA

Education, Audiovisual & Culture
Executive Agency





Histopathology. 2013 May;62(6):827-39.

Step	Special considerations
1. Pre-analytic steps	<ul style="list-style-type: none"> Define clear objectives Design experiment to answer the specific research question Identify and request any ethical and consent Consider histotechnical and technical steps that may be a subsequent problem Write a protocol for the study, including timing, storage and data analysis methods
2. Tissue bank	<ul style="list-style-type: none"> Review existing tissue banks by expert histopathologist to ensure that bank For prospective collection, write protocol to ensure standardized tissue handling Complete profile of collected tissue and record study progression to ensure potential bias identified by timing issues
3. Tissue bank protocols	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies
4. Consistent Tissue Bank	<ul style="list-style-type: none"> Design experiment, protocol for the bank Consider 'member' open to specific sub-studies for high density tissue, consider grid design with sub-study Review bank protocols in the grid and, if using multiple samples per grid, each grid has a code in a different region bank If sampling heterogeneity from specific regions bank to normal regions consider representative 'biopsy'
5. Consistent Tissue	<ul style="list-style-type: none"> Review research bank to ensure sufficient quality Review sufficient density from steps of tissue to the research aim Consider timing and growth rates from the research bank to determine growth Consider timing and growth rates for high density tissue Design bank studies of open Tissue Bank is supported by an expert histopathologist
6. Tissue analysis	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies
7. Perform histology on other than Optimal histology	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies
8. Review histology	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies
9. Perform statistical analysis	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies
10. Share data	<ul style="list-style-type: none"> Review bank policies and ensure are consistent with aims of research not limited by expert histopathologist Consider bank policies open and data sampling that is consistent with the underlying research question and histopathology of tissue Obtain appropriate consent for inclusion in each Tissue Bank Complete additional parts of protocol for this tissue bank for histopathological studies



Lifelong Learning Programme



Tissue heterogeneity as a pre-analytical source of variability

Tumor heterogeneity as an example

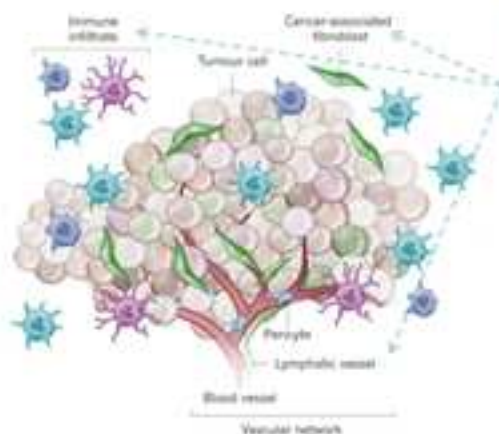


Ana Justino



Tumor heterogeneity

"...tumors are more than insular masses of proliferating cancer cells. Instead, they are **complex tissues composed of multiple distinct cell types** that participate in heterotypic interactions with one another." (Hanahan D. and Weinberg RA, Cell, 2011; 144: 646-74)



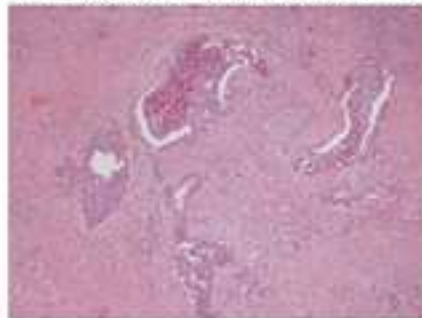
**Tumor micro-environment
heterogeneity**

Adapted from Jurekta M.R., et al. Nature, 2013; 501: 346-54.

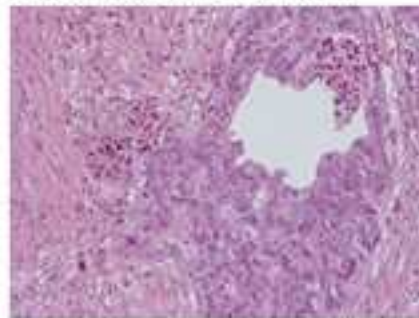
Tumor heterogeneity

Tumor micro-environment heterogeneity

- Morphological evaluation of a case with 10% of tumor cells



Amplification 50X



Amplification 200X

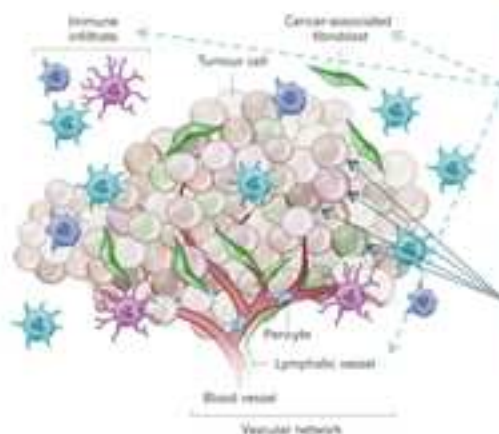
Pre-analytical source of variability

Macrodissection

Microdissection

Tumor heterogeneity

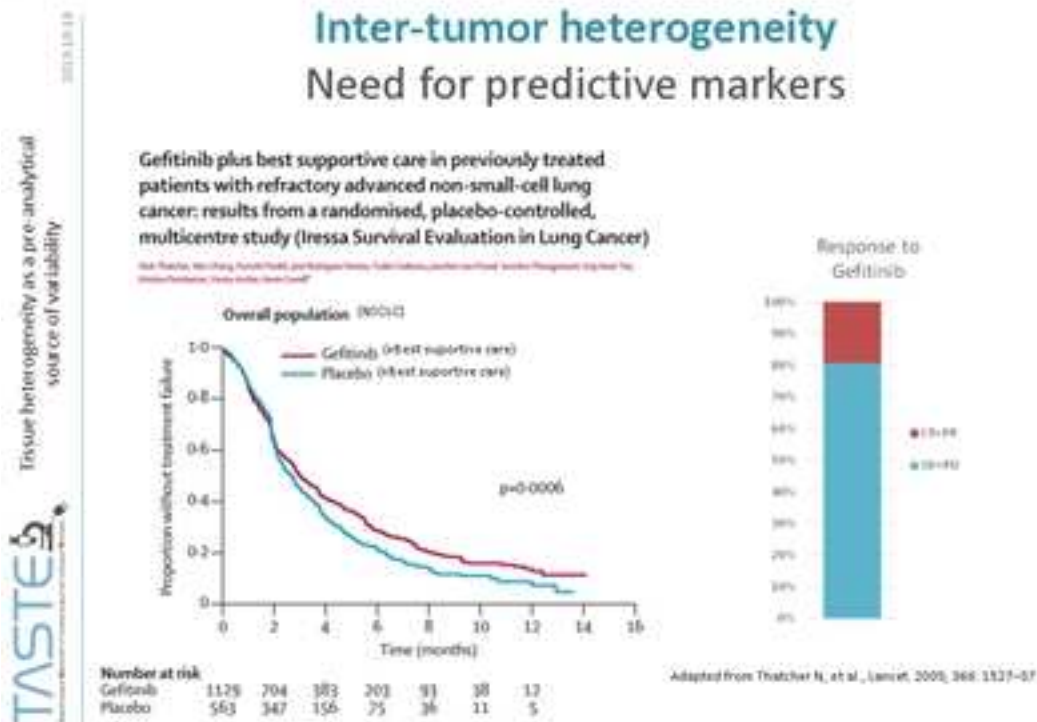
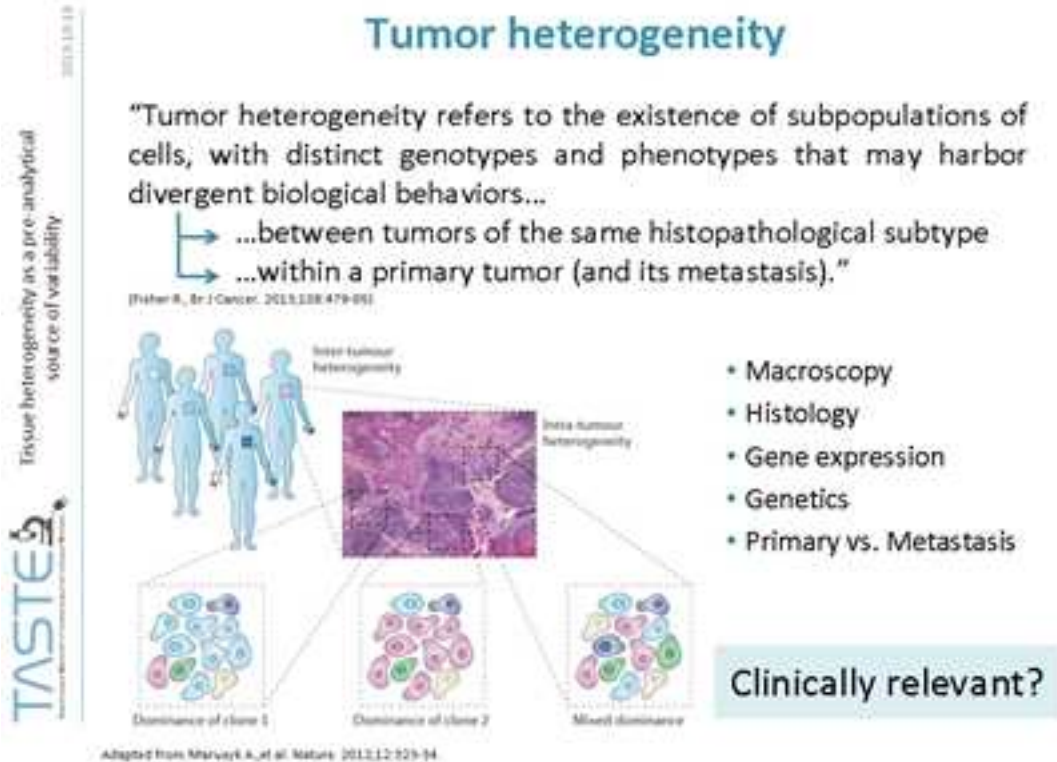
"...tumors are more than insular masses of proliferating cancer cells. Instead, they are **complex tissues composed of multiple distinct cell types** that participate in heterotypic interactions with one another."



Tumor micro-environment heterogeneity

Subpopulations of cancer cells
Tumor Heterogeneity

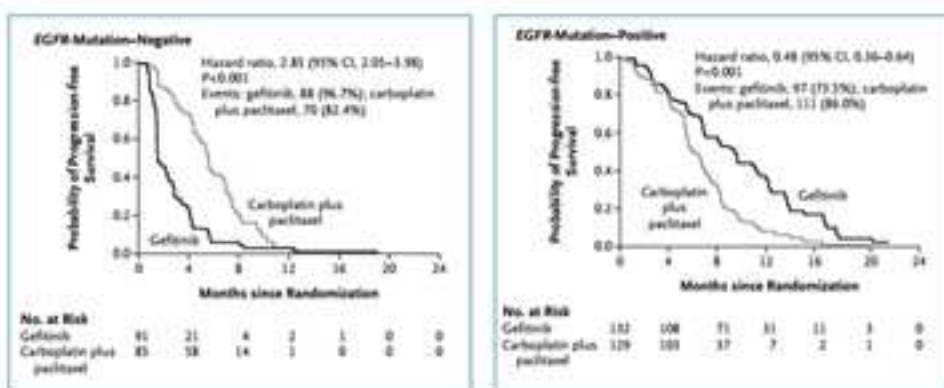
Adapted from Junttilä M.R., et al. Nature 2013;502: 546-54.



Inter-tumor heterogeneity Need for predictive markers

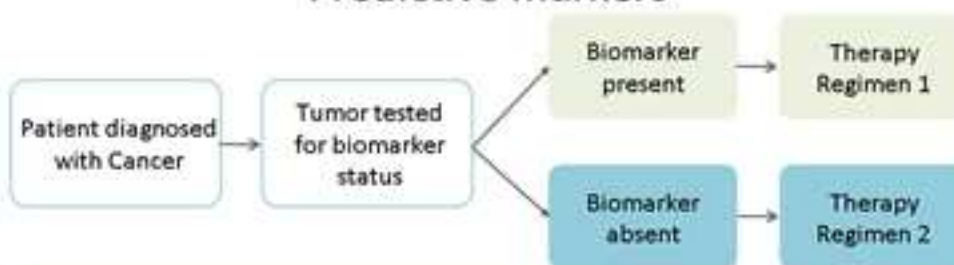
Gefitinib or Carboplatin-Paclitaxel in Pulmonary Adenocarcinoma

Sung S. Huh, M.D., Yi-Liang Wu, M.D., F.A.C.S., Sumatra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D.,
Da-Feng Chou, M.D., Nagesh Desai, M.D., Ph.D., Pongpon Lertsakulwong, M.D., Rattana Inan, M.D.,
Benjamin Margolis, M.D., Ph.D., F.Z.C.P., Yuhya Ichimura, M.D., Yutaka Horiuchi, M.D., Ph.D.,
Yuchiro Ohta, M.D., Ph.D., Jin-Jong Yeng, M.D., Rukhsana Chaudhury, M.D., Hyeon Jeong, M.D.,
James C. Duffy, M.Sc., Clara L. Watkins, M.Sc., Alison A. Arshaw, F.A.C.S., and Masahiro Furukawa, M.D., Ph.D.

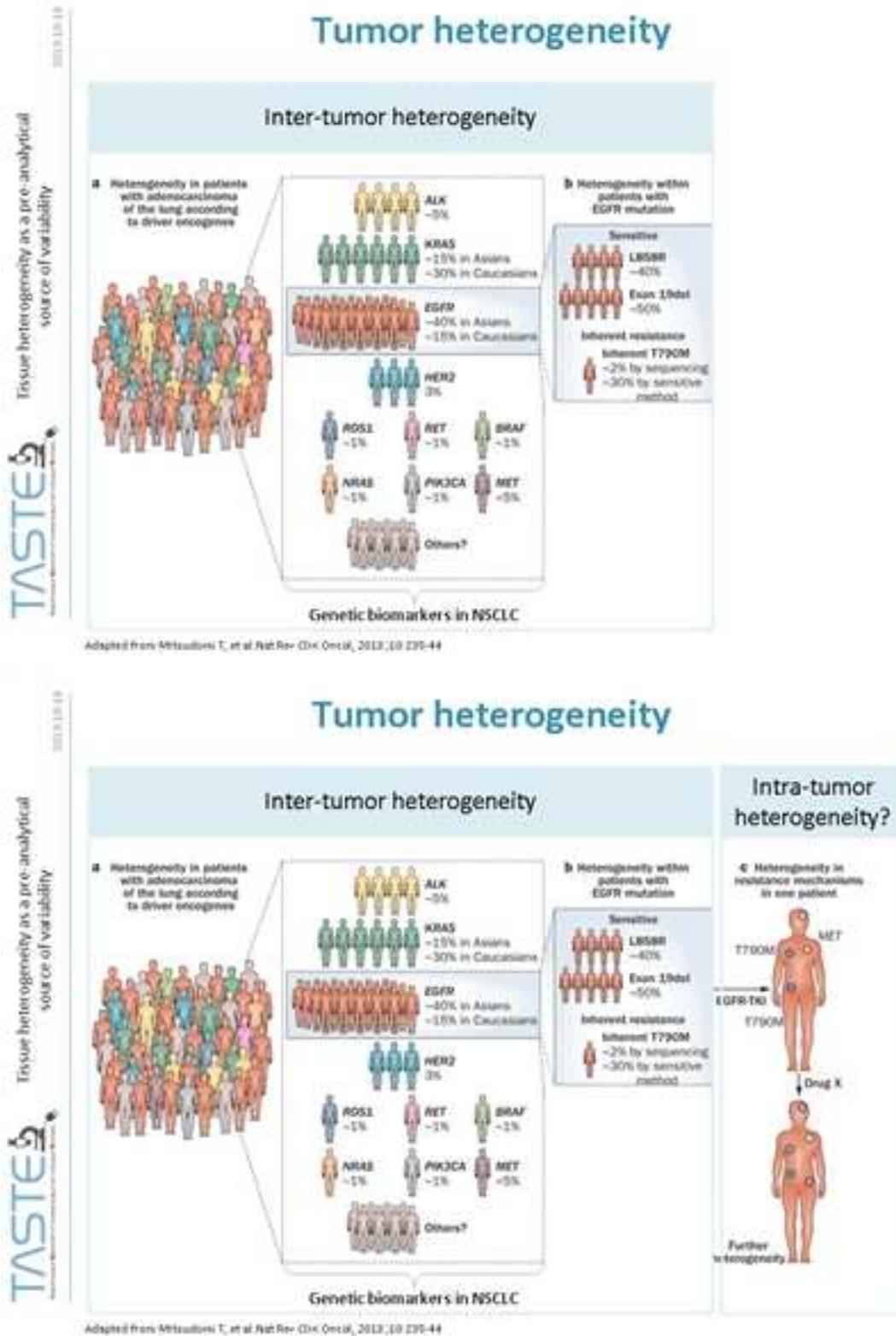


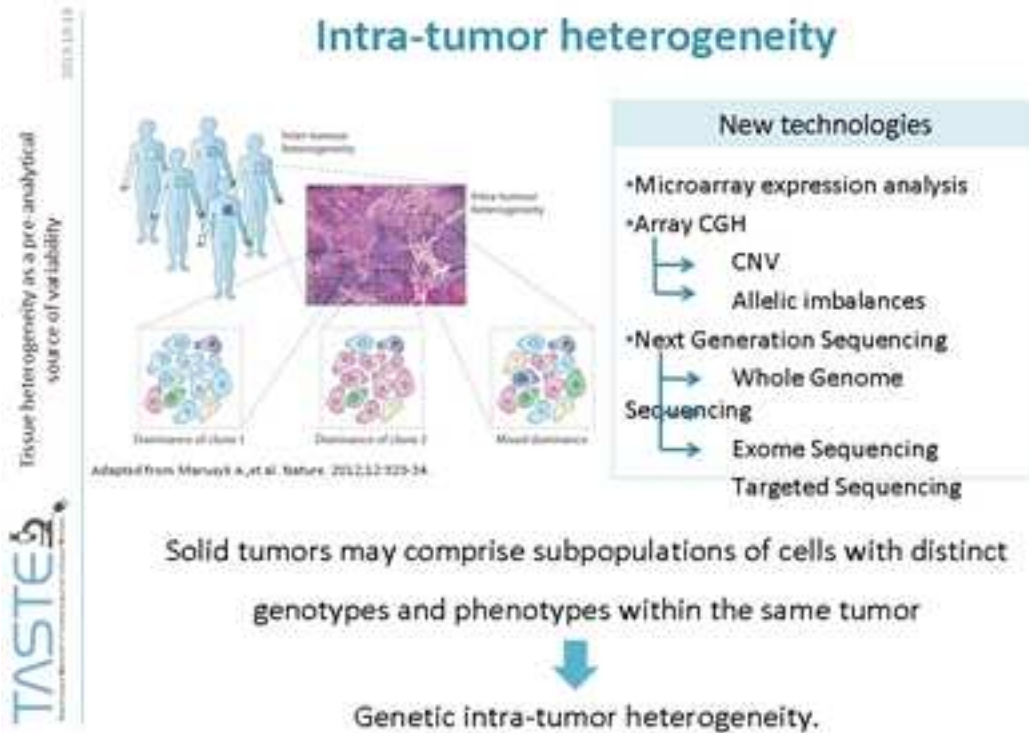
Adapted from Huh SS, et al., N Engl J Med. 2009;361:947-57

Inter-tumor heterogeneity Predictive markers



Cancer	Drug	Biomarker	% eligible
Breast	Trastuzumab	HER2 gene amplification	30%
Lung	Gefitinib/Erlotinib	EGFR mutation	12%
Lung	Crizotinib	EML4-ALK translocation	5%
Colon	Cetuximab/Panitumumab	KRAS mutation	55%
CML	Imatinib	BCR-ABL translocation	95%
GIST	Imatinib	KIT/PDGFR mutation	90%
Gastric	Trastuzumab	HER2 gene amplification	20%
Melanoma	Vemurafenib	BRAF mutation	42%





2013.10.10

Tissue heterogeneity as a pre-analytical source of variability

Intra-tumor heterogeneity

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

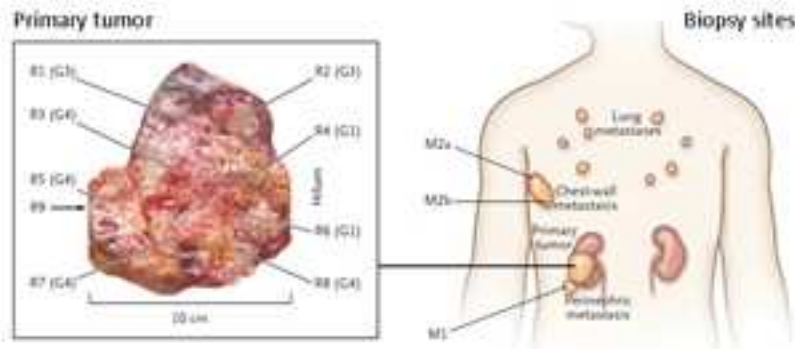
Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Mario Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endersfield, Dip.Math., Eva Gromova, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Philpott, B.Sc., Sharmistha Begum, M.Sc., Neil O. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Kayvan Raine, M.Sc., Calli Lister, B.Sc., Claudio R. Santos, Ph.D., Mahesh Nishadani, H.N.C., Aron C. Elland, Ph.D., Bradley Spencer Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zulfan Seifian, M.D., Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.

Does single tumor biopsy samples portrait mutational tumor landscapes?

TASTE

Intra-tumor heterogeneity

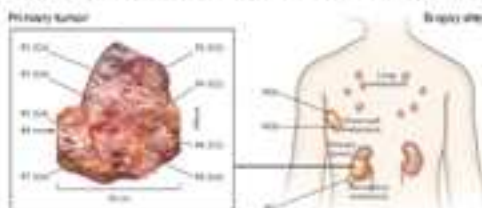


Adapted from Gerlinger et al. N Engl J Med. 2012;366:889-92.

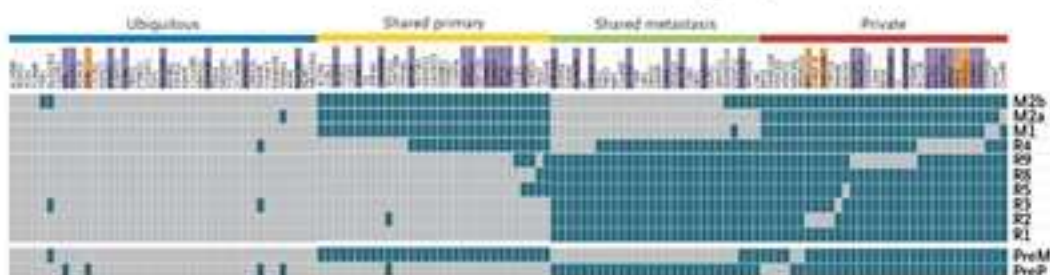
Whole-exome sequencing of multiple tumor samples from primary and metastatic lesions

Intra-tumor heterogeneity

Regional distribution of mutations

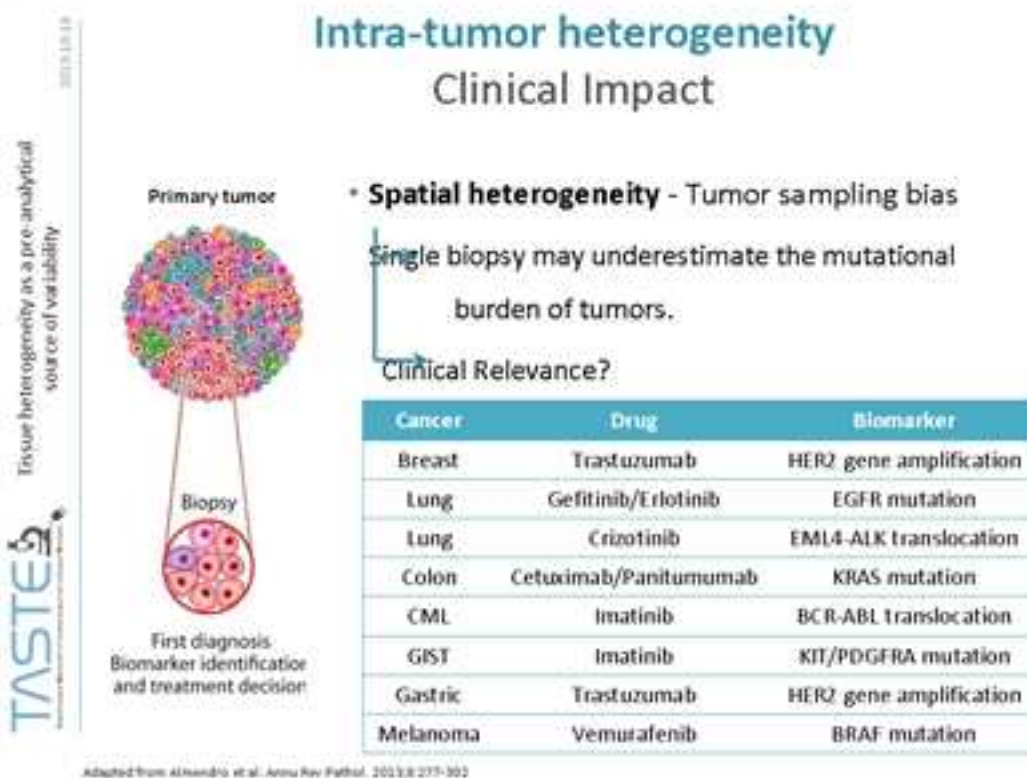
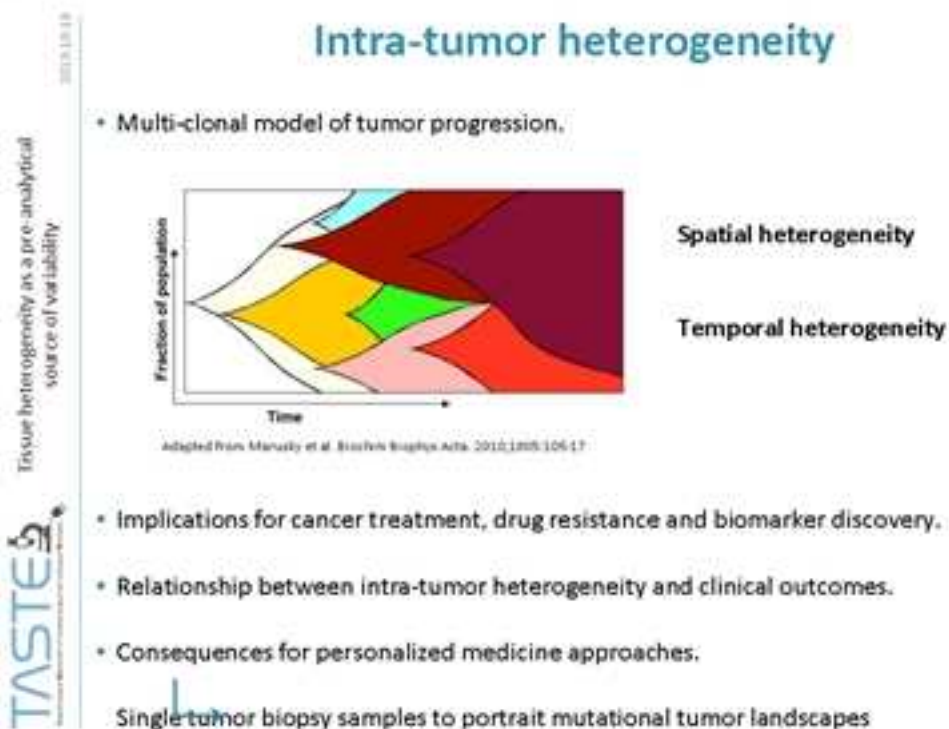


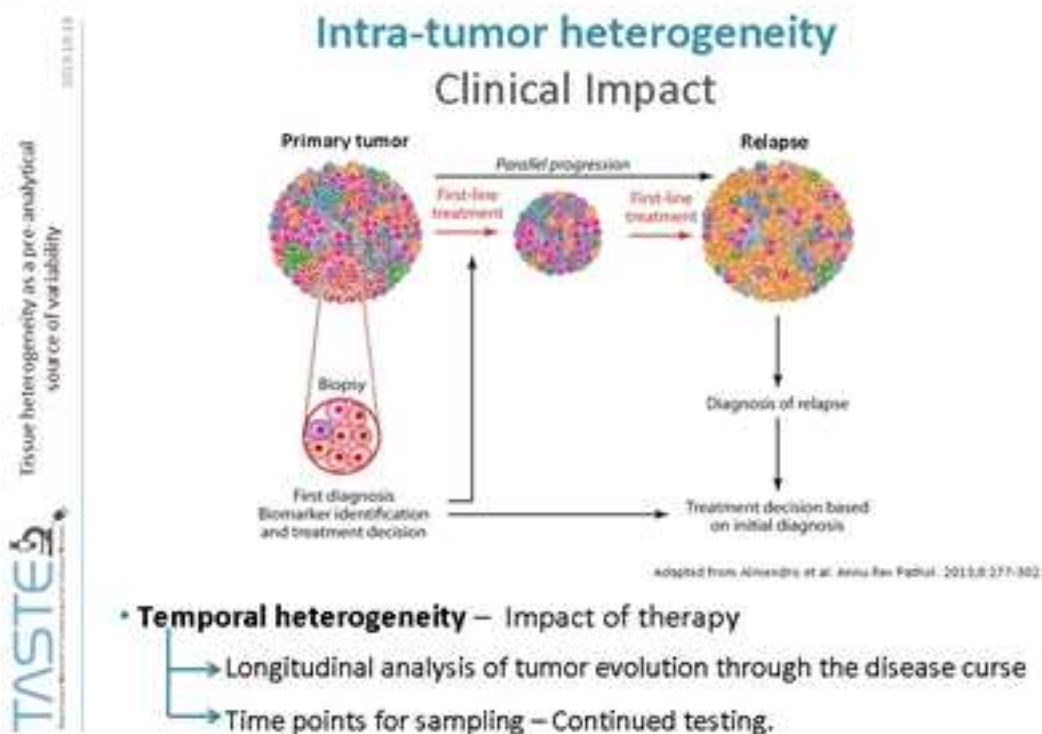
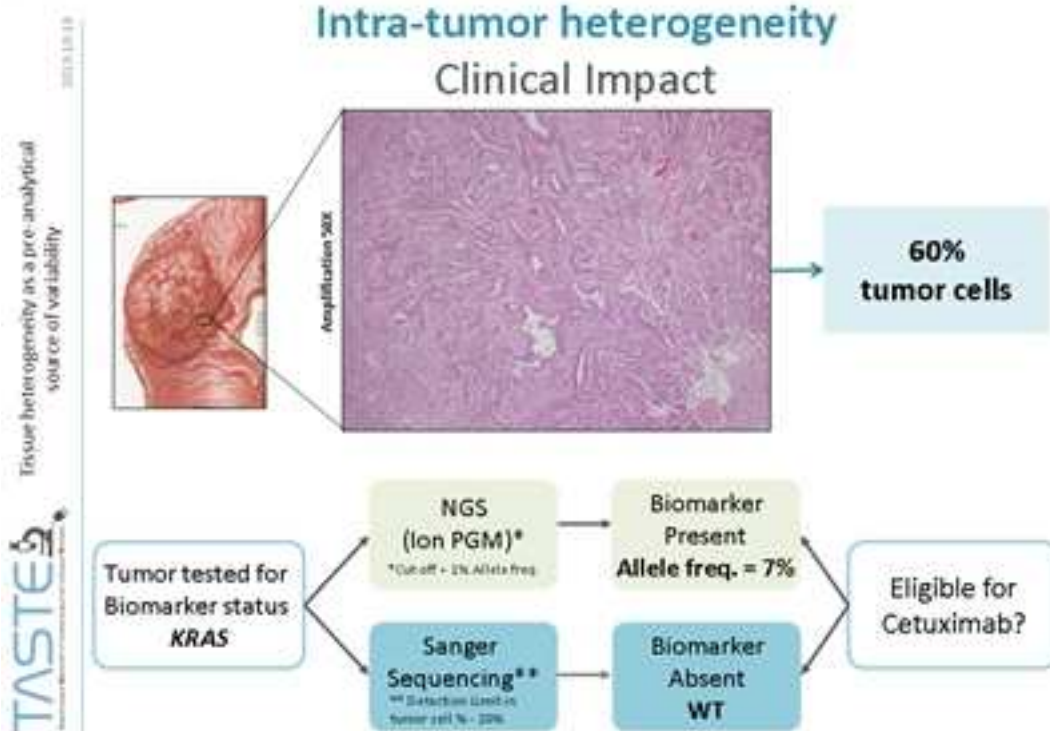
- 133 different somatic mutations
- Average 70 mutations per sample
- Only 34% of all mutations were ubiquitous...
- ...or only 25% of the mutations fall outside the two main groups



Extensive intra-tumor heterogeneity

Adapted from Gerlinger et al. N Engl J Med. 2012;366:889-92.



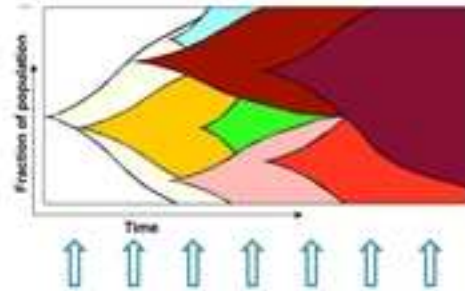


Intra-tumor heterogeneity Clinical Impact

Histology	Adeno	Adeno	Adeno
Genotype	L858R TP53	L858R TP53 T790M	L858R TP53
EGFR TKI status	Sensitive	Resistant	Sensitive
Tumor burden			
Treatment	Chemo	Erlotinib	Chemo
Timeline	2007	2008	2009

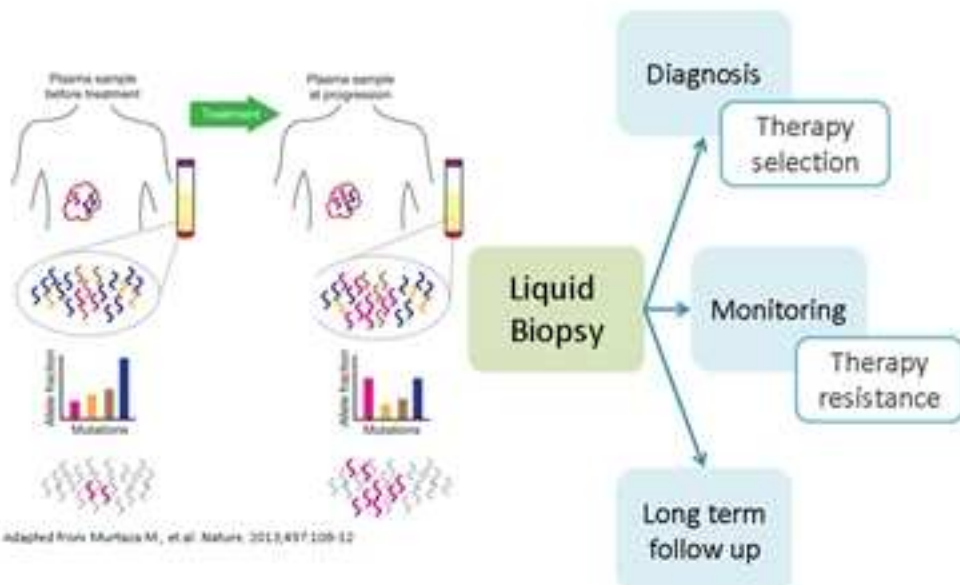
Adapted from Sequist V, et al. *N Engl J Med* 2012;367:1903-14

Optimized care may require continued biopsies and genotyping

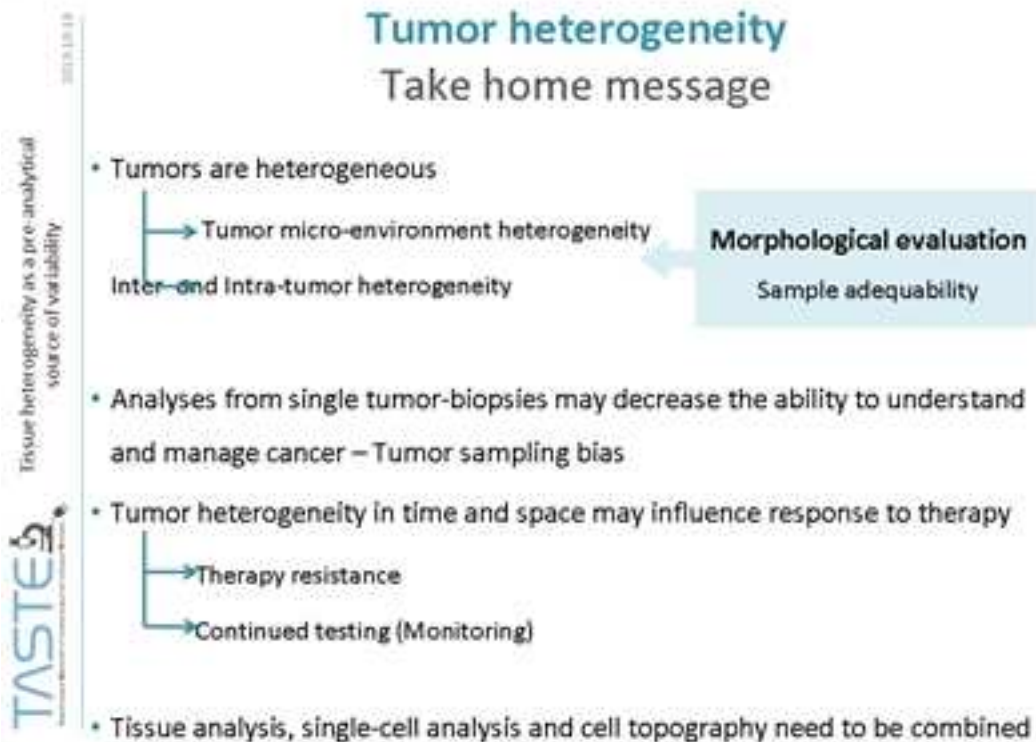


Adapted from Maruyama et al. *Breast Cancer* 2012;19:105-17

Intra-tumor heterogeneity Future direction?



Adapted from Maruyama M, et al. *Nature* 2012;497:109-12



***Tissue heterogeneity as a pre-analytical
source of variability***

Tumor heterogeneity as an example



Ana Justino

