La integración de la patología molecular en el entorno digital

ENRIQUE DE ALAVA

IBiS
INSTITUTODEBIOMEDICINADESEVILLA

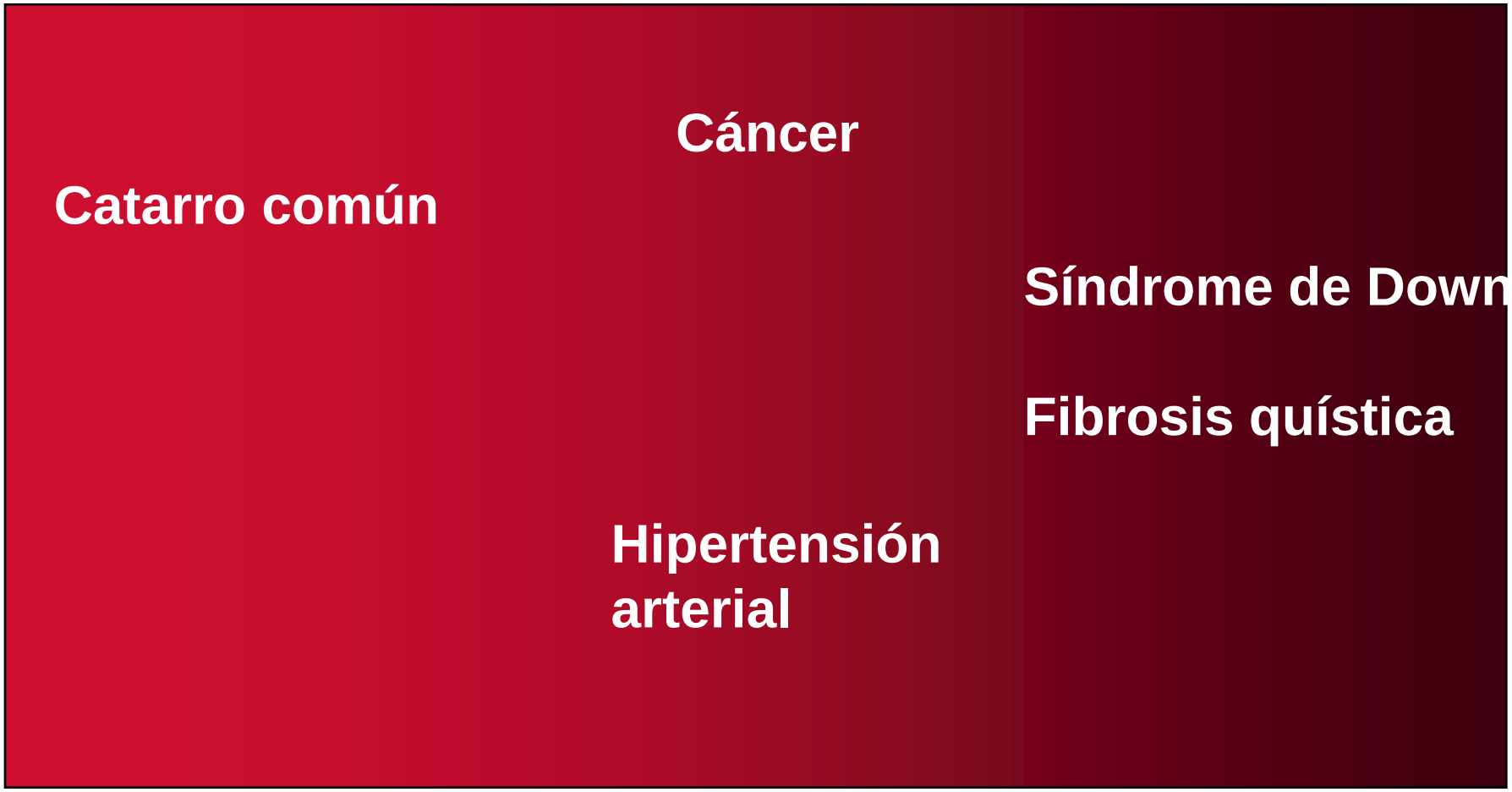
Guión

- Un entorno cambiante
- Necesitamos mirar de manera diferente.
- Algunos retos.
  - *La IHQ y patología molecular morfológica*
  - *Medir la heterogeneidad*
  - *Desarrollando biomarcadores*
  - *Gigantes con pies de barro*
  - *La patología molecular no morfológica*
- Hacia una visión global

Momento de cambio

- Progreso tecnológico rápido con producción masiva de datos
- Progreso en bioinformática-computación
- Biología de sistemas frente a reduccionismo
- Acceso a la personalización (genoma)
- Globalización

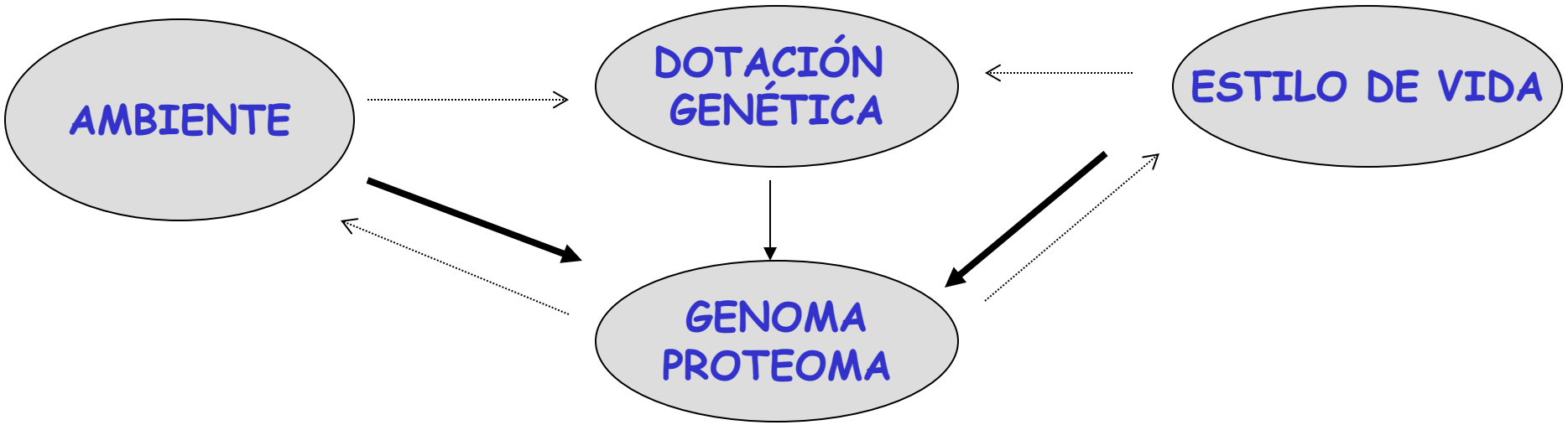
Enfermedad



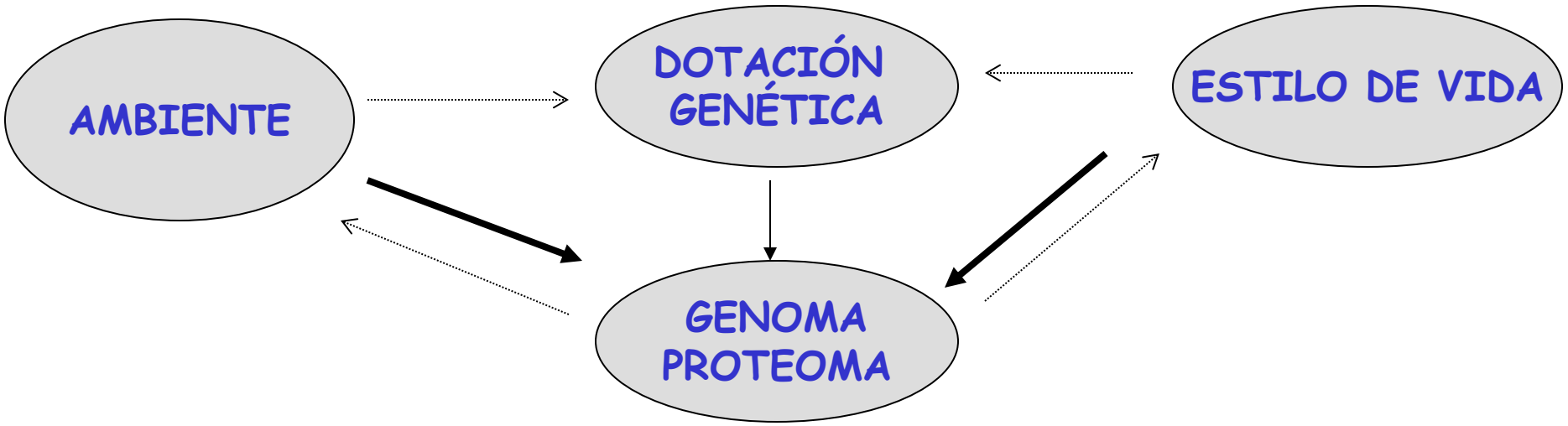
Ambiente
Estilo de vida

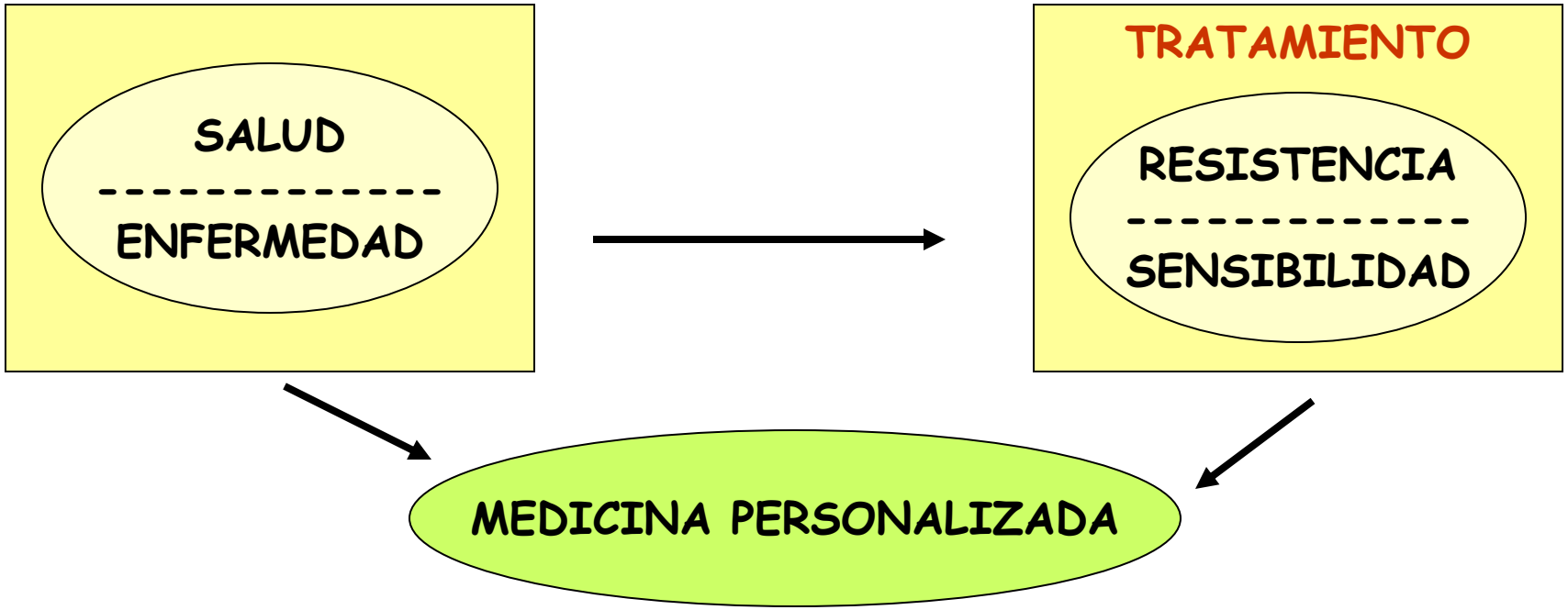
Genes

BIOMEDICINA Y ASISTENCIA SANITARIA: CONCEPTO ACTUAL



BIOMEDICINA Y ASISTENCIA SANITARIA: CONCEPTO ACTUAL

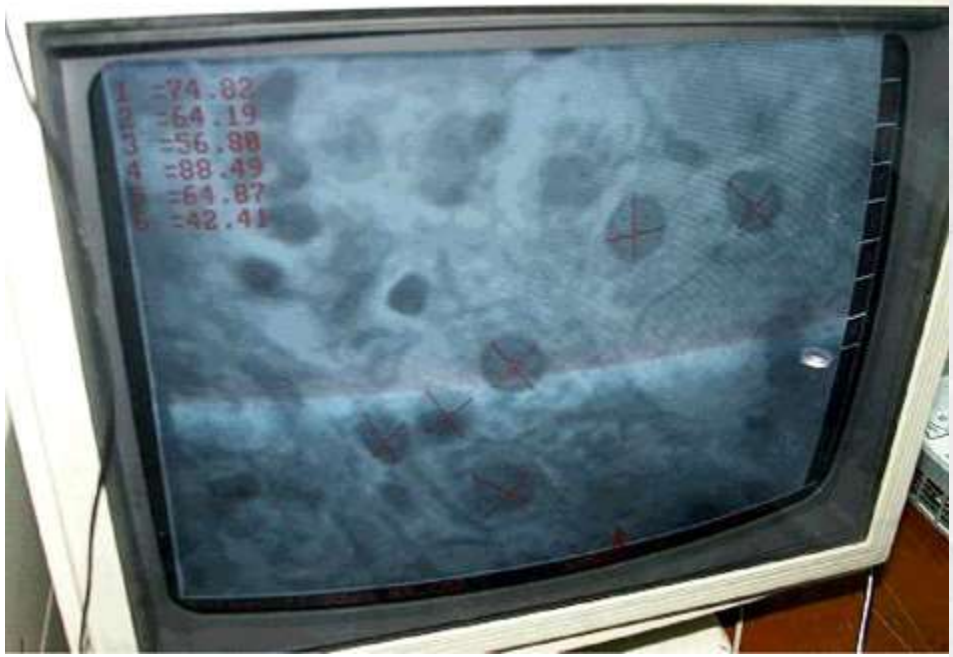


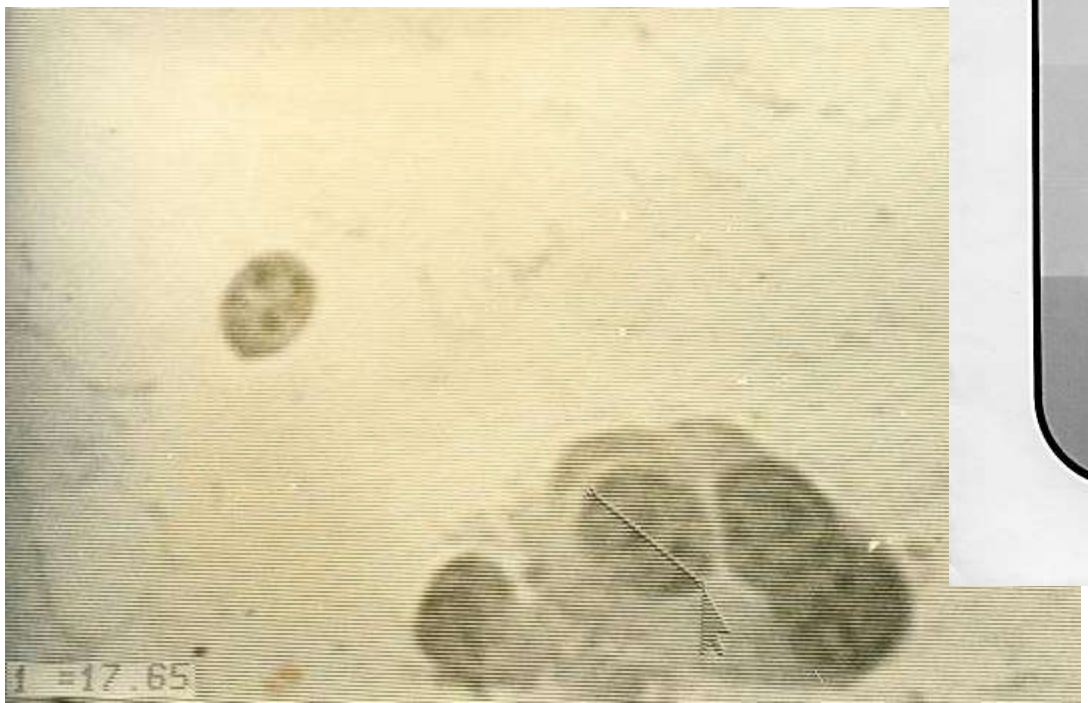


Biomarcadores (p.ej. en cáncer)

- Moléculas en cantidades anormales en fluidos o tejidos de algunos pacientes.
- Varían en distintos tipos de cáncer.
- Utilidad:
  - Diagnóstica
  - Pronóstica
  - Predictiva
  - Monitorización
- Se utilizan junto con otras pruebas.

www.nci.nih.gov





PUBLICACION OFICIAL DE LA SOCIEDAD ESPAÑOLA DE ANATOMIA PATOLOGICA

Patología

SUMARIO

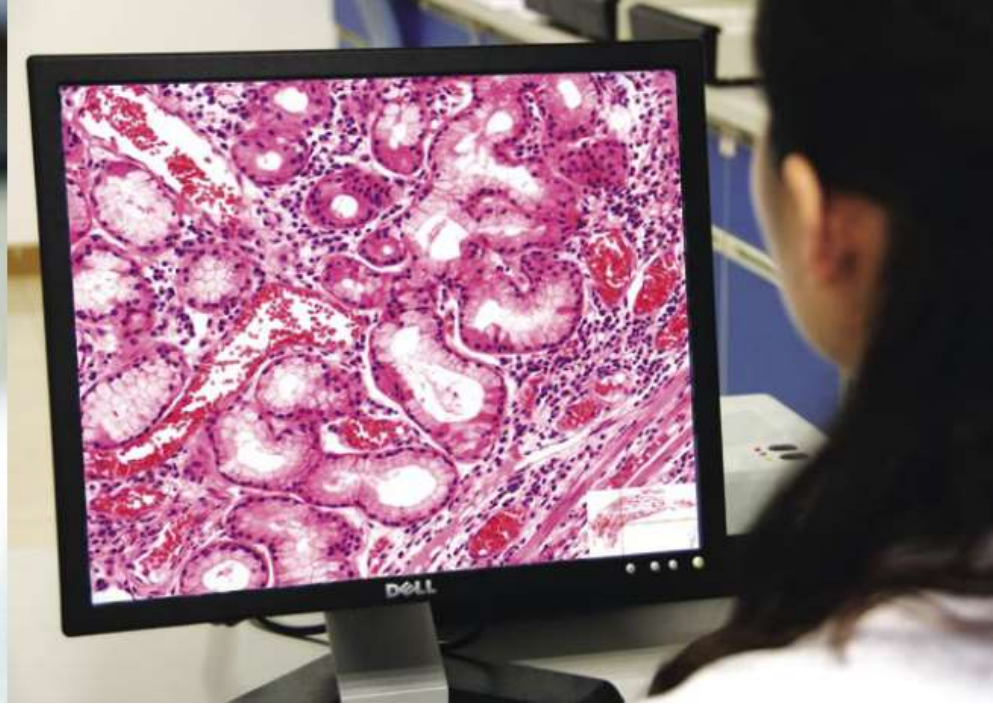
VOLUMEN 20 • NUMERO 1 • ENERO-MARZO 1987

www.conganat.org

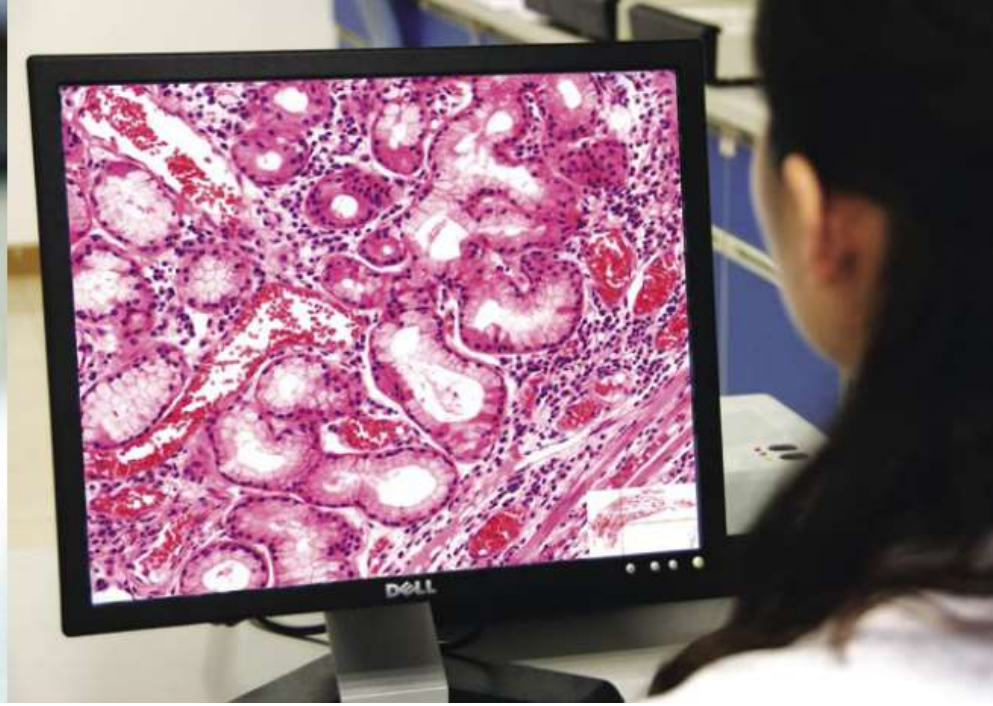

Sun
Medscape ®
http://www.medscape.com

Patología molecular en el entorno digital

1. La patología molecular descansa en los tejidos (en buena parte y por el momento).
2. Terapias dirigidas, basadas en la presencia/ausencia de biomarcadores en tejidos.
3. Desarrollo tecnológico (digitalización de portas completos).



El patólogo ya no tiene por qué estar donde están los portas



Impacto en la ordenación del trabajo

- Integración
- Colaboración
- Externalización
- Centralización

La interpretación visual de la IHQ cromogénica es subjetiva y tendente a errores

Journal of Pathology
J Pathol 2009; **218**: 285–291
Published online 29 January 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2527

Review Article

Do we see what we think we see? The complexities of morphological assessment

Peter W Hamilton,[1]* Paul J van Diest,[2] Richard Williams[3] and Anthony G Gallagher[4]
[1]*Centre for Cancer Research and Cell Biology, Queen's University of Belfast, Belfast, UK*
[2]*Department of Pathology, UMC Utrecht, Utrecht, The Netherlands*
[3]*Department of Anatomical Pathology, St Vincen's Hospital and The University of Melbourne, Melbourne, Australia*
[4]*National Surgical Training Centre, Royal College of Surgeons Ireland, Dublin, Ireland*

- Ilusiones ópticas
- Variabilidad en la comprensión del significado de algunas claves visuales
- Variabilidad en las estrategias espaciales de búsqueda
- Variabilidad en las estrategias cognitivas
- Variabilidad en el peso que se da a un rasgo concreto
- Variabilidad en umbrales de detección
- Variabilidad de la identificación de eventos infrecuentes
- Capacidad de procesamiento simultáneo limitada
- Cansancio
- El papel de la intuición y lo difícil que es trasmitirla
- …

Reproducibility of p53 Immunohistochemistry in Bladder Tumors[1]

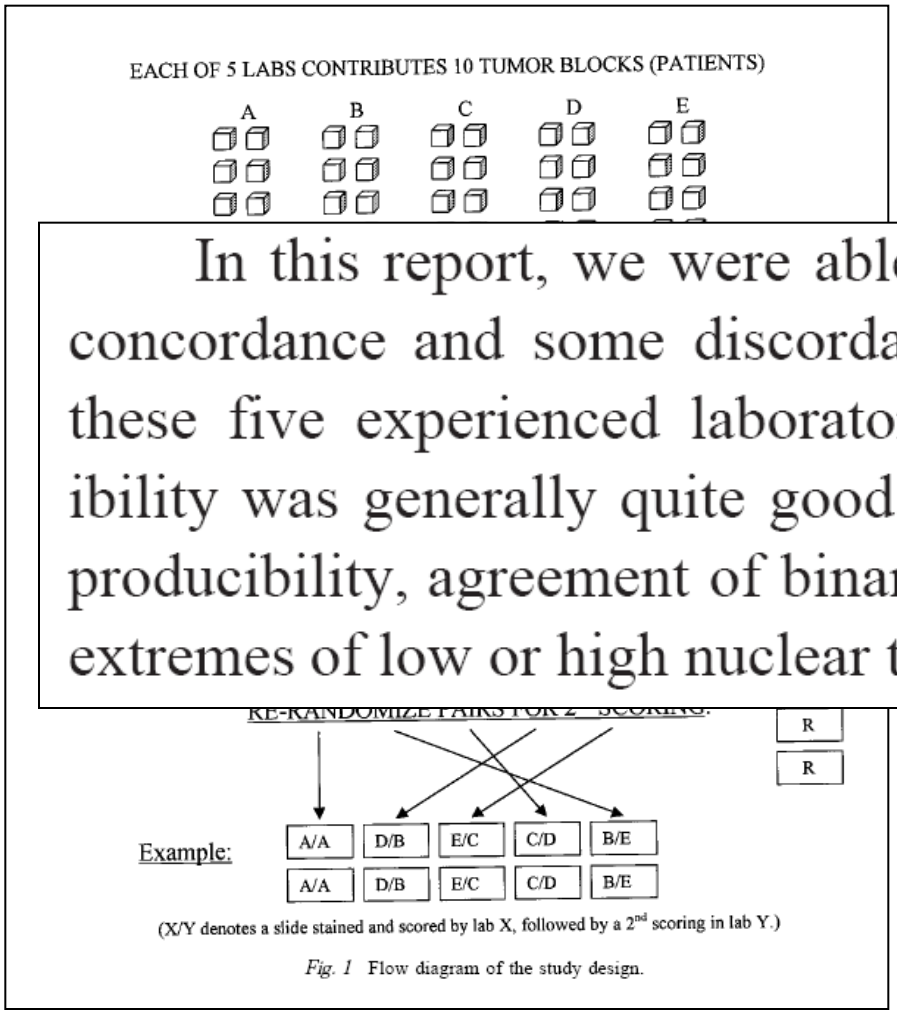


Fig. 1 Flow diagram of the study design.

In this report, we were able to identify both considerable concordance and some discordance among the p53 assays in these five experienced laboratories. Intralaboratory reproducibility was generally quite good. Regarding interlaboratory reproducibility, agreement of binary assessments was good at the extremes of low or high nuclear tumor cell staining percentages.

Study of Interlaboratory Reliability and Reproducibility of Estrogen and Progesterone Receptor Assays in Europe

Am J Clin Pathol 2001;115:44-58

Documentation of Poor Reliability and Identification of Insufficient Microwave Antigen Retrieval Time as a Major Contributory Element of Unreliable Assays

Anthony Rhodes, PhD,[1] Bharat Jasani, PhD,[2] Andre J. Balaton, MD,[3] Diana M. Barnes, DSc,[4] Elizabeth Anderson, PhD,[5] Lynda G. Bobrow, FRCPath,[6] and Keith D. Miller, FIBMS[1]

Key Words: Immunohistochemistry; Estrogen receptors; Progesterone receptors; Technical validation; Microwave antigen retrieval

Inadequate assay sensitivity, with subsequent weak staining, was the main cause of poor and variable results by laboratories using microwave antigen retrieval; too short a heating time was identified as the principal contributory factor. Extension of the heating time resulted in significant improvement regardless of all other variables in the immunohistochemical protocol. Continual participation in EQA is an effective means for identifying and ameliorating variables that influence the reliability of immunohistochemical assays for predictive markers, thereby assisting in technical validation and standardization.

An International Ki67 Reproducibility Study

Results Intralaboratory reproducibility was high (
only moderate (central staining: ICC = 0.71
Geometric mean of Ki67 values for each la
ing and 6.1% to 30.1% with local staining
region selection, counting method, and s
gave more consistent results than visual e

Conclusions Substantial variability in Ki67 scoring was
Ki67 values and cutoffs for clinical decisior
izing scoring methodology because analyt

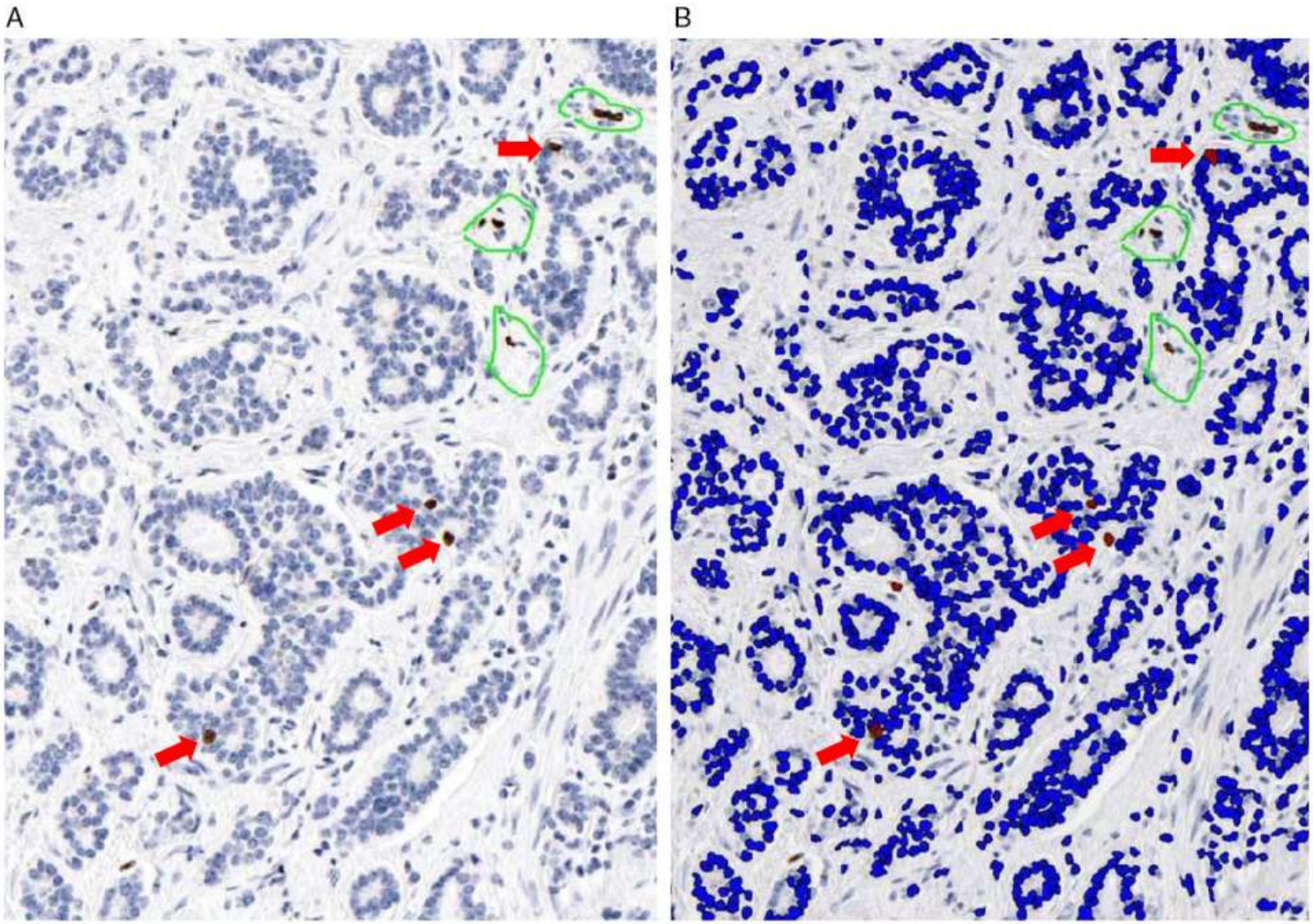
J Natl Cancer Inst;2013;105:1897–1906

	Scoring
Lab	**Scoring method**
Lab A	Visual estimate 1% increments (<10%), 5% increments (10%–30%), 10% increments (>30%)
Lab B	Visual estimate (1% increments)
Lab C	Visual estimate 1% increments (<5%), 5% increments (10%–40%), 10% increments (>40%)
Lab D	Visual estimate (1% increments)
Lab E	Counting 500 cells using a hemocytometer; any level of stain is positive
Lab F	Counting ~200 cells using a keypad Web application
Lab G	Visual estimate 10% increments (exceptions made for 1% and 95%: 28 cases scored in 1% increments and one case scored as 95%)
Lab H	Counting entire invasive tumor in each core using a 10 × 10 grid in a ×10 eyepiece graticule

Objective Quantification of the Ki67 Proliferative Index in Neuroendocrine Tumors of the Gastroenteropancreatic System

A Comparison of Digital Image Analysis With Manual Methods

(*Am J Surg Pathol* 2012;36:1761–1770)



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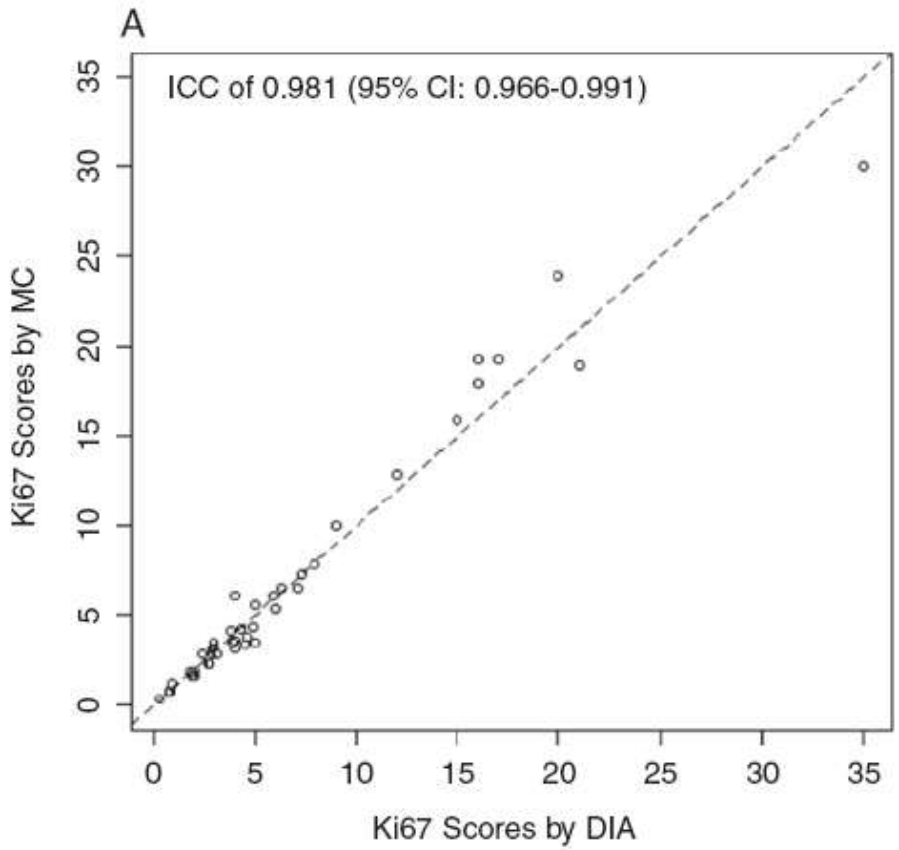
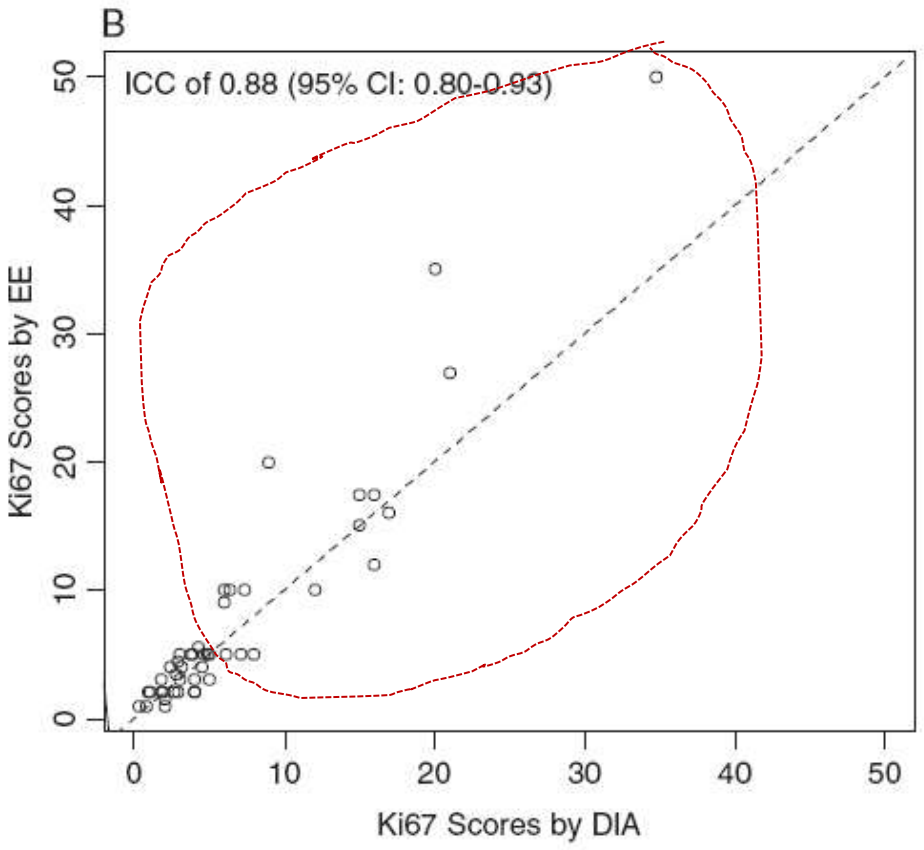
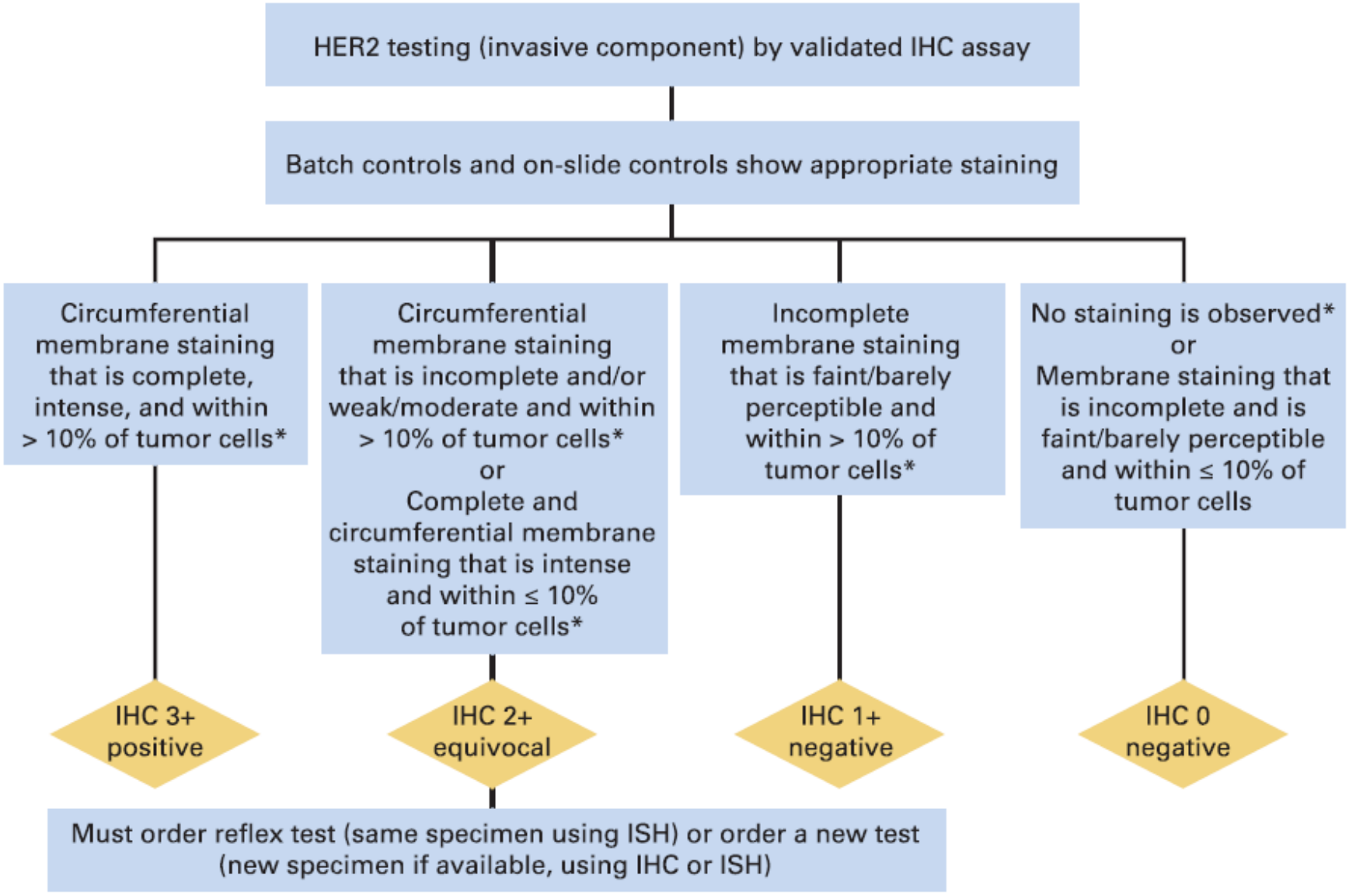




ASCO/CAP HER2 Testing Guideline Update–



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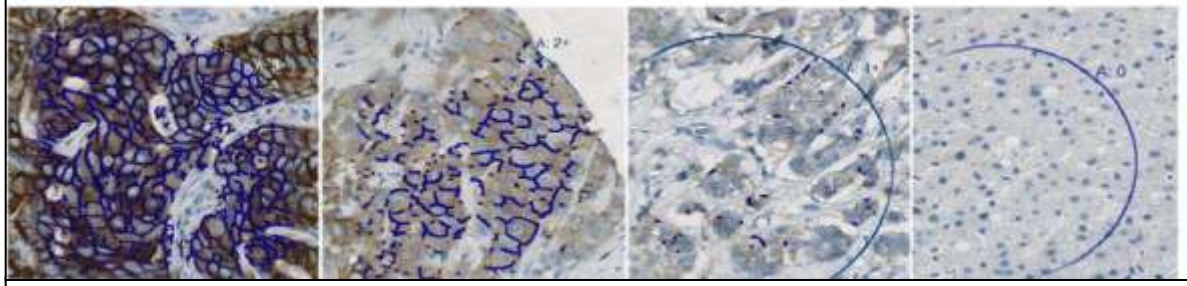
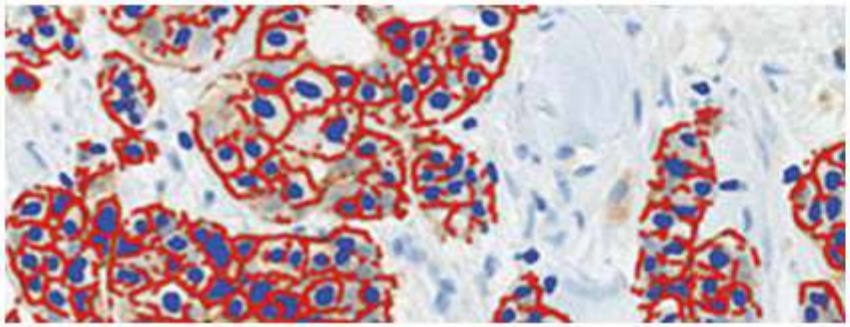
HER2-CONNECT™





The VENTANA Companion Algorithm HER2 (4B5) image analysis software

Roche

Aperio®
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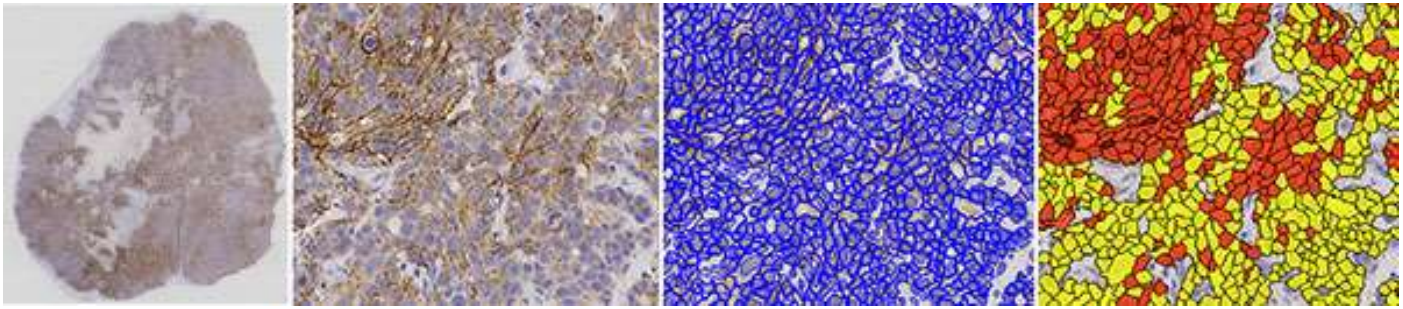
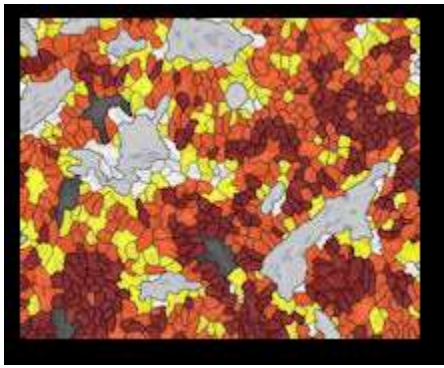
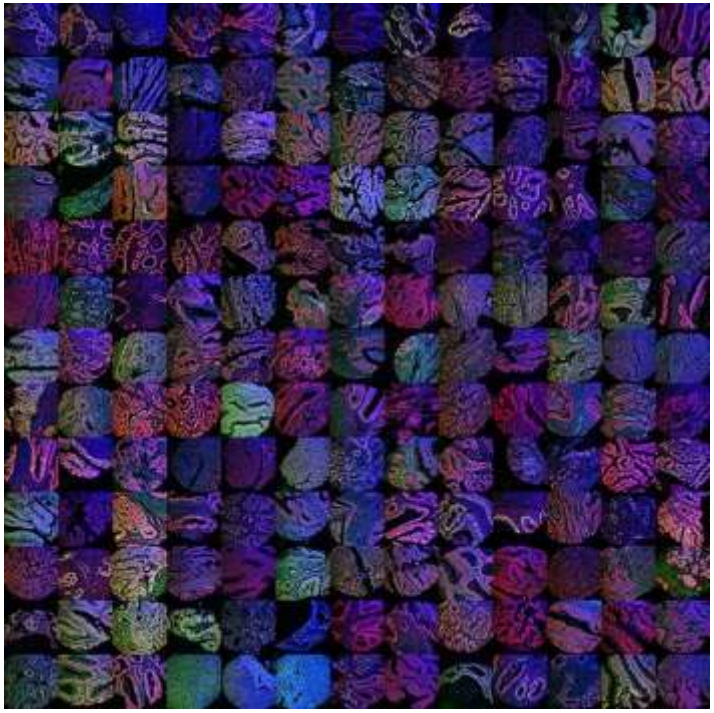
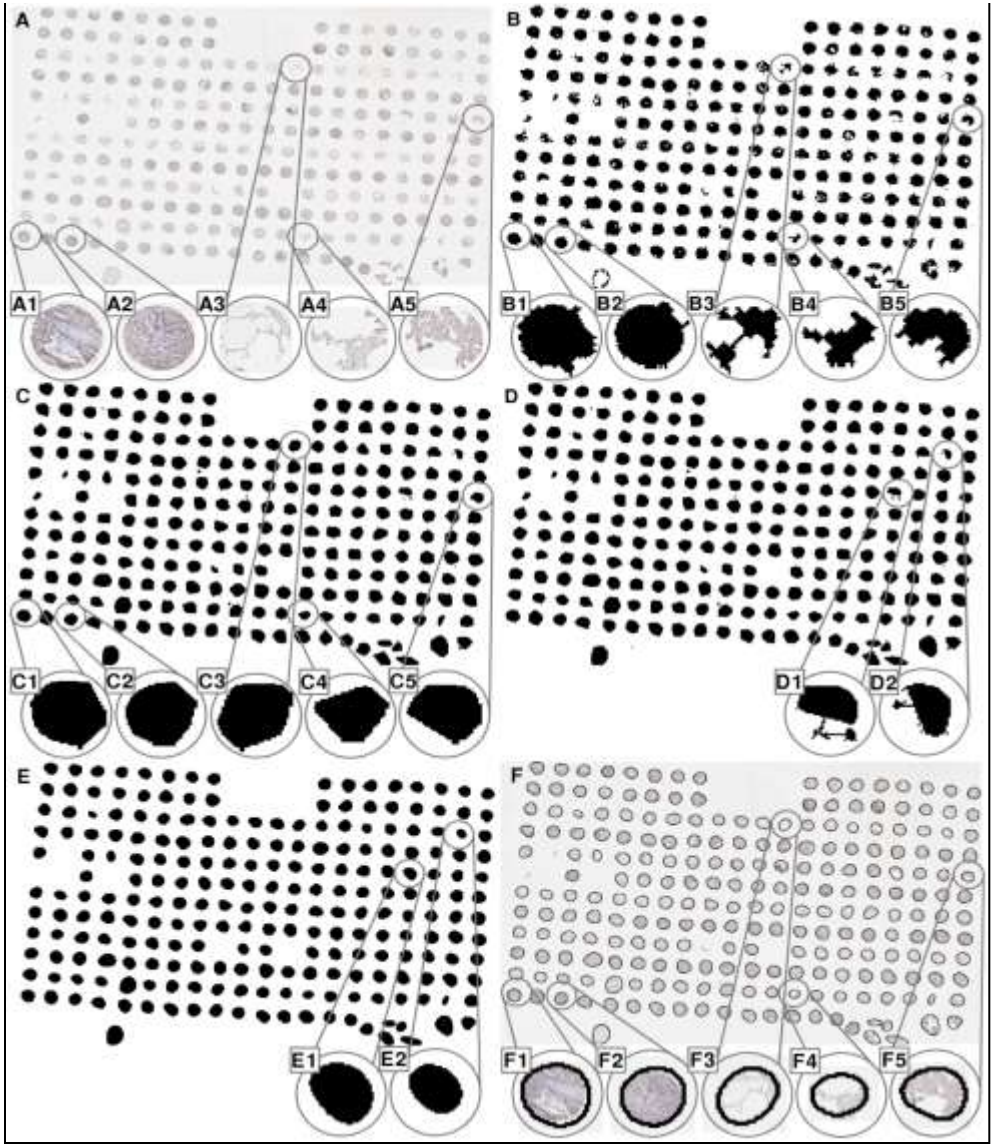


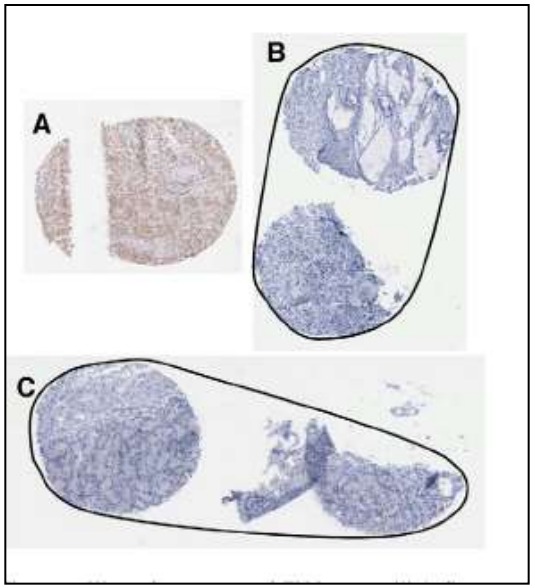


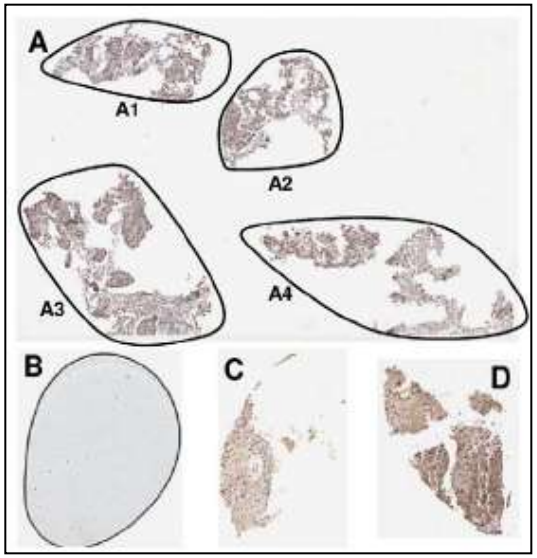


A TMA De-Arraying Method for High Throughput Biomarker Discovery in Tissue Research

PLoS ONE 6(10): e26007. doi:10.1371/journal.pone.0026007







jove Journal of Visualized Experiments www.jove.com

Video Article

A Next-generation Tissue Microarray (ngTMA) Protocol for Biomarker Studies

Inti Zlobec[1], Guido Suter[1], Aurel Perren[1], Alessandro Lugli[1]

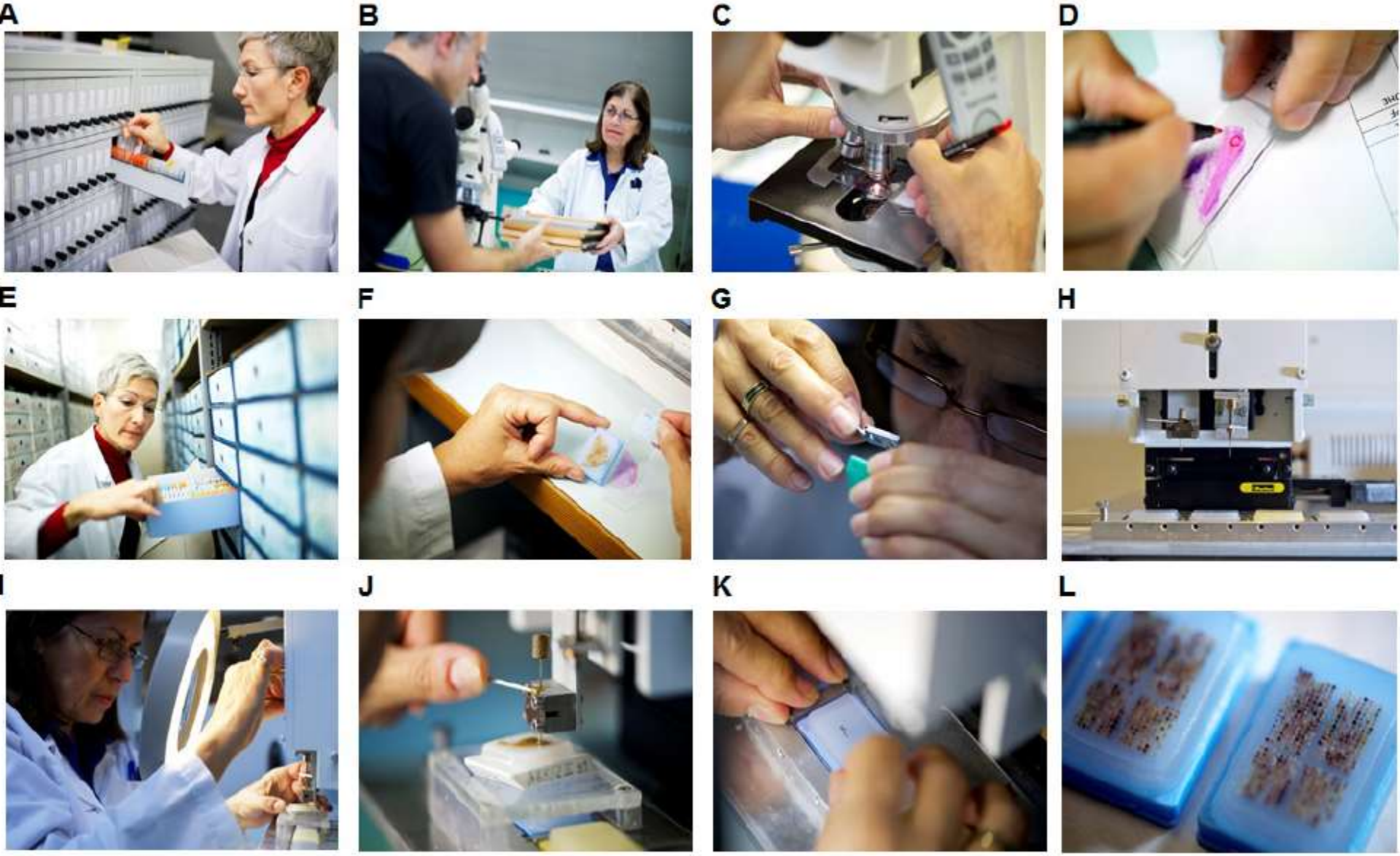
[1]Institute of Pathology, University of Bern

Correspondence to: Inti Zlobec at inti.zlobec@pathology.unibe.ch

URL: http://www.jove.com/video/51893
DOI: doi:10.3791/51893

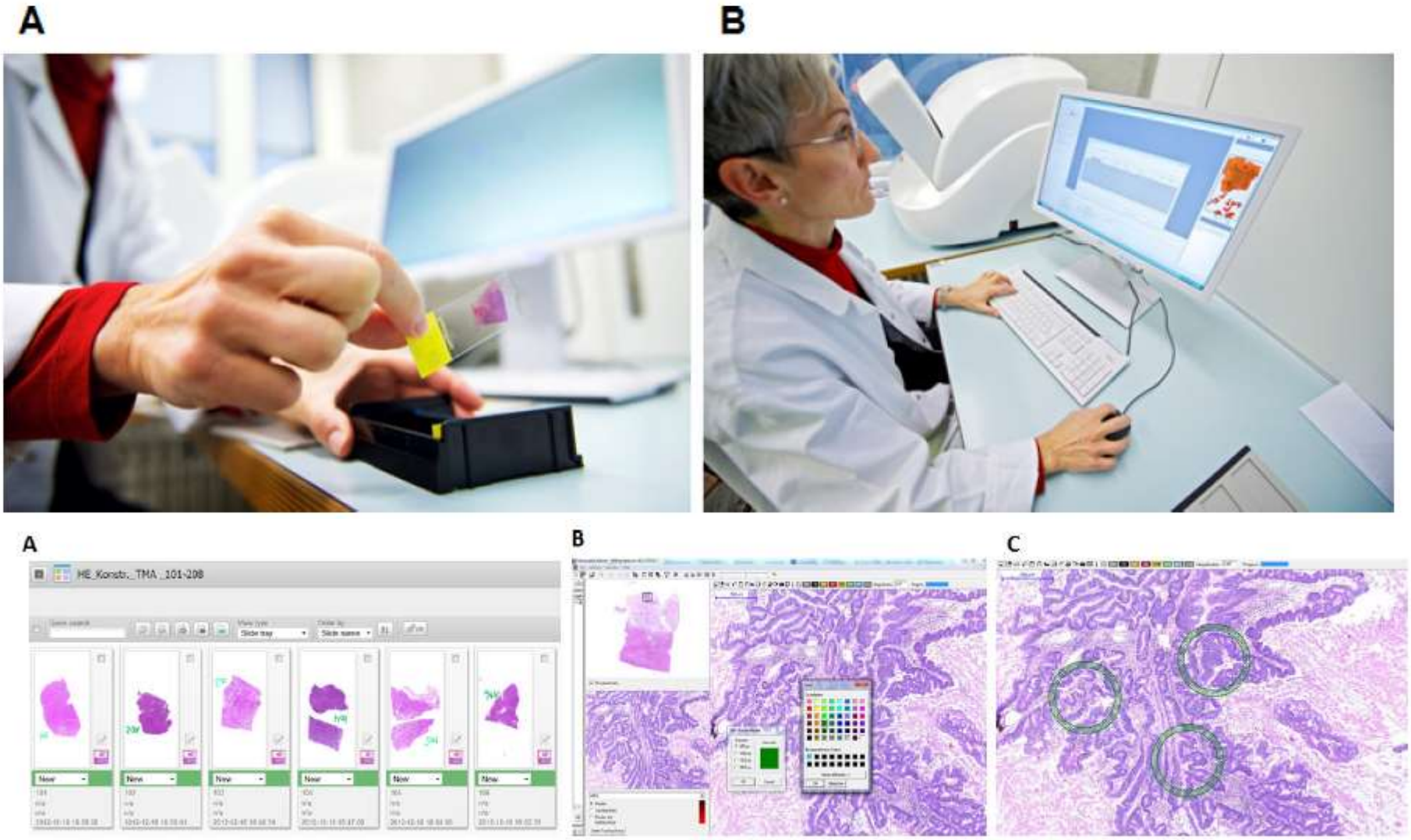
http://www.jove.com/video/51893/

La manera tradicional de hacer un TMA

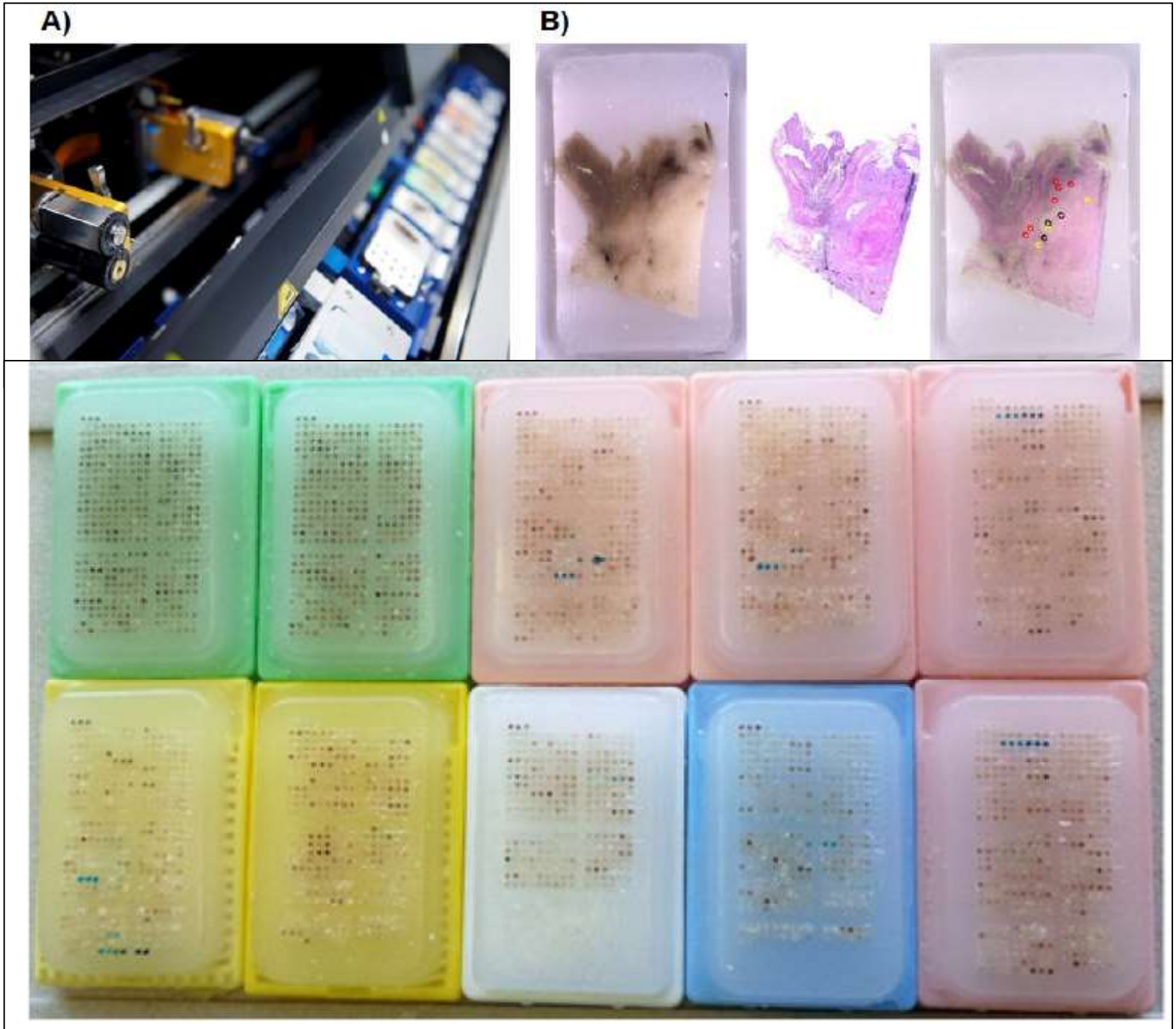


http://www.jove.com/video/51893/

TMAs de nueva generación (1)



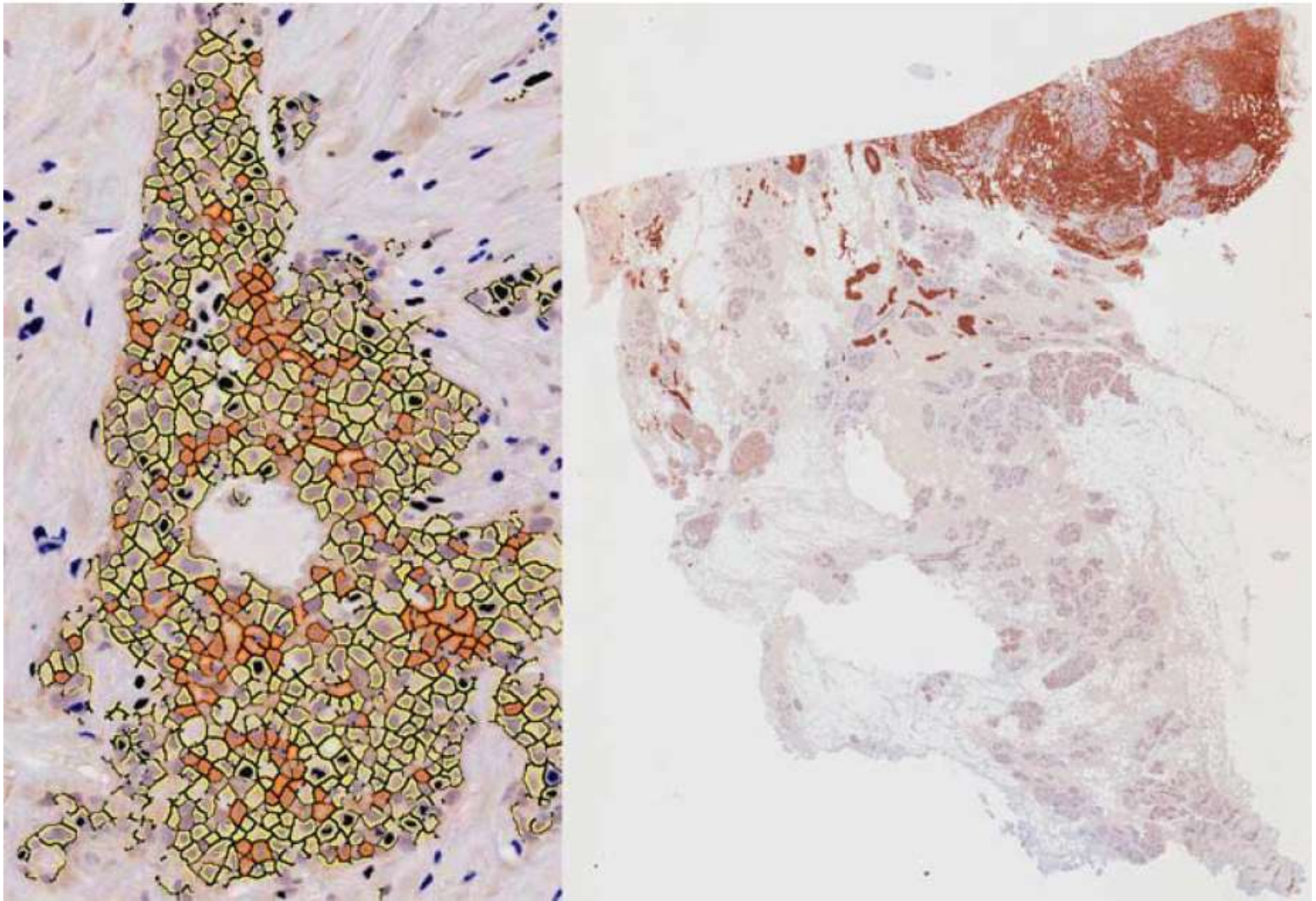
http://www.jove.com/video/51893/


A)
B)

Evaluating tumor heterogeneity in immunohistochemistry-stained breast cancer tissue

Steven J Potts[1], Joseph S Krueger[1], Nicholas D Landis[1], David A Eberhard[2], G David Young[1], Steven C Schmechel[3] and Holger Lange[1]

Laboratory Investigation (2012) **92,** 1342–1357;



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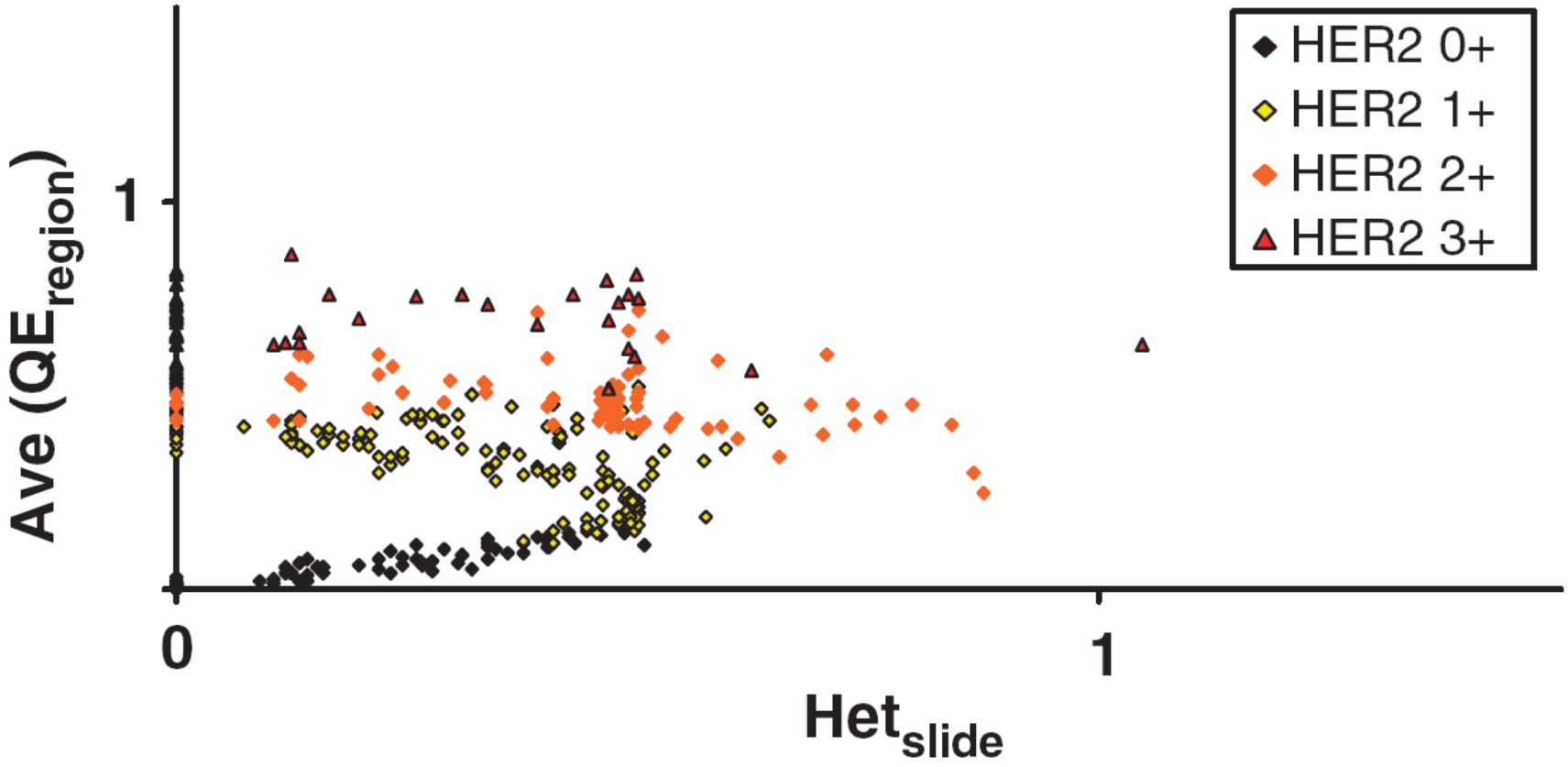
Laboratory Investigation (2012) **92,** 1342–1357;



Problemas con la detección cromogénica

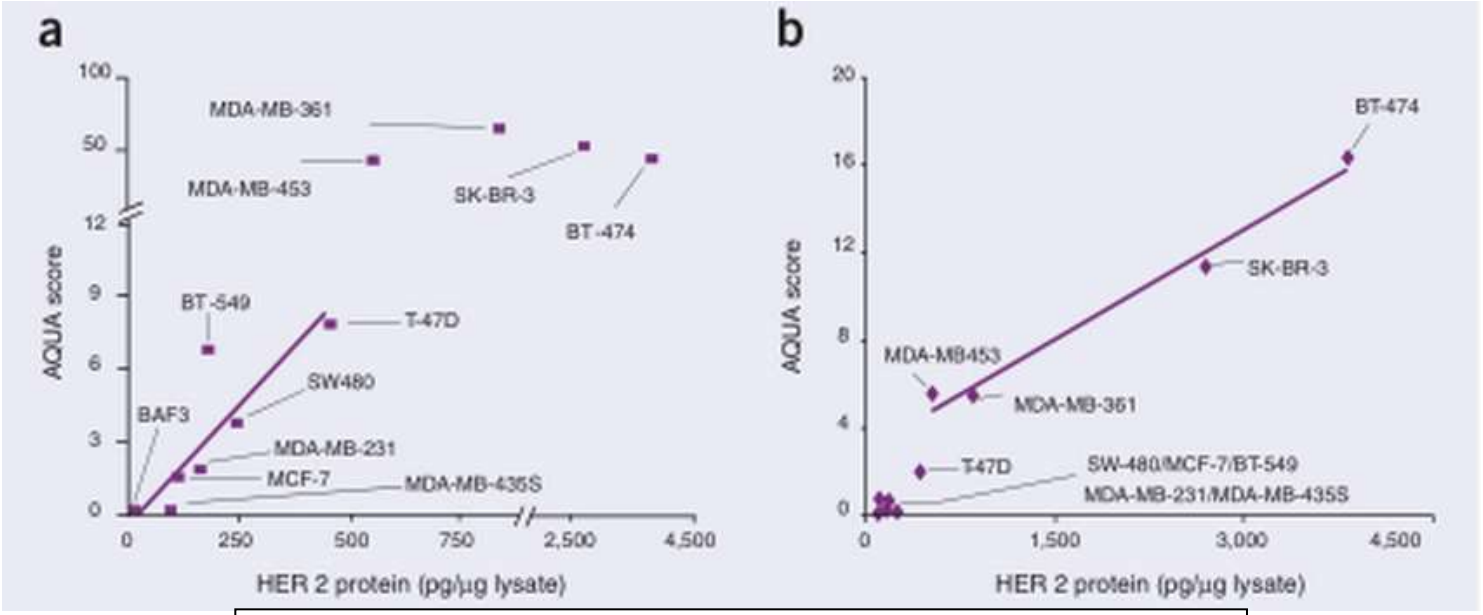
Cromógeno

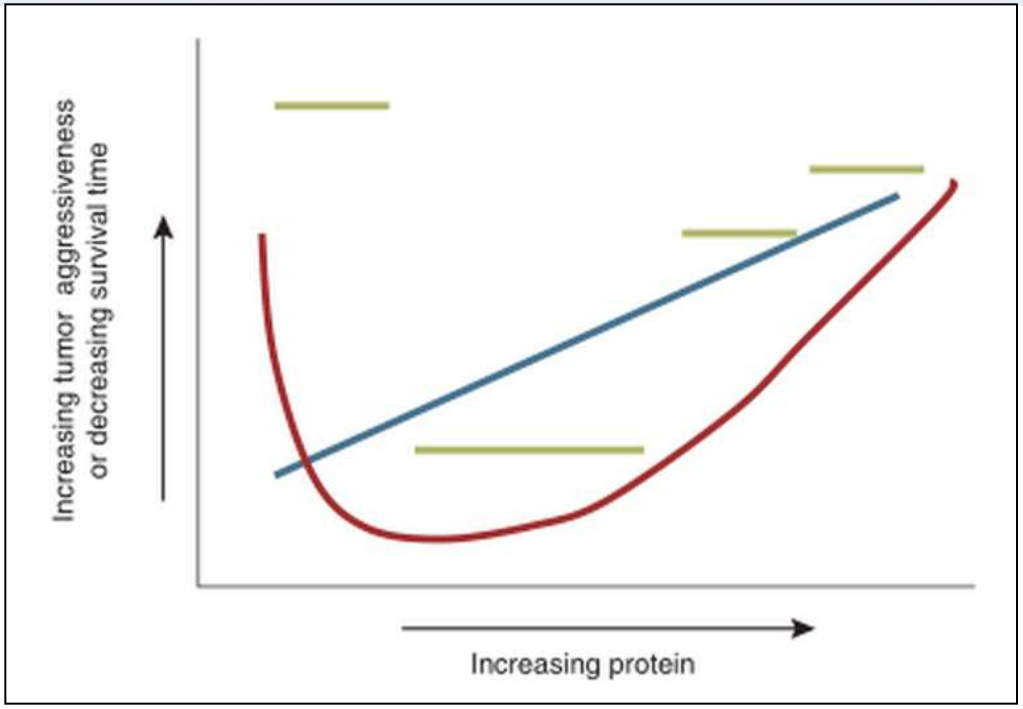
- Difícil cuantificación
- Rango dinámico bajo
- Propiedades espectrales que cambian con la intensidad

Fluorescencia

- Relación lineal fotones/intensidad del pixel
- Detección de múltiples marcadores
- Se pierde con el tiempo

Hamilton et al. Methods 2014 (en prensa)

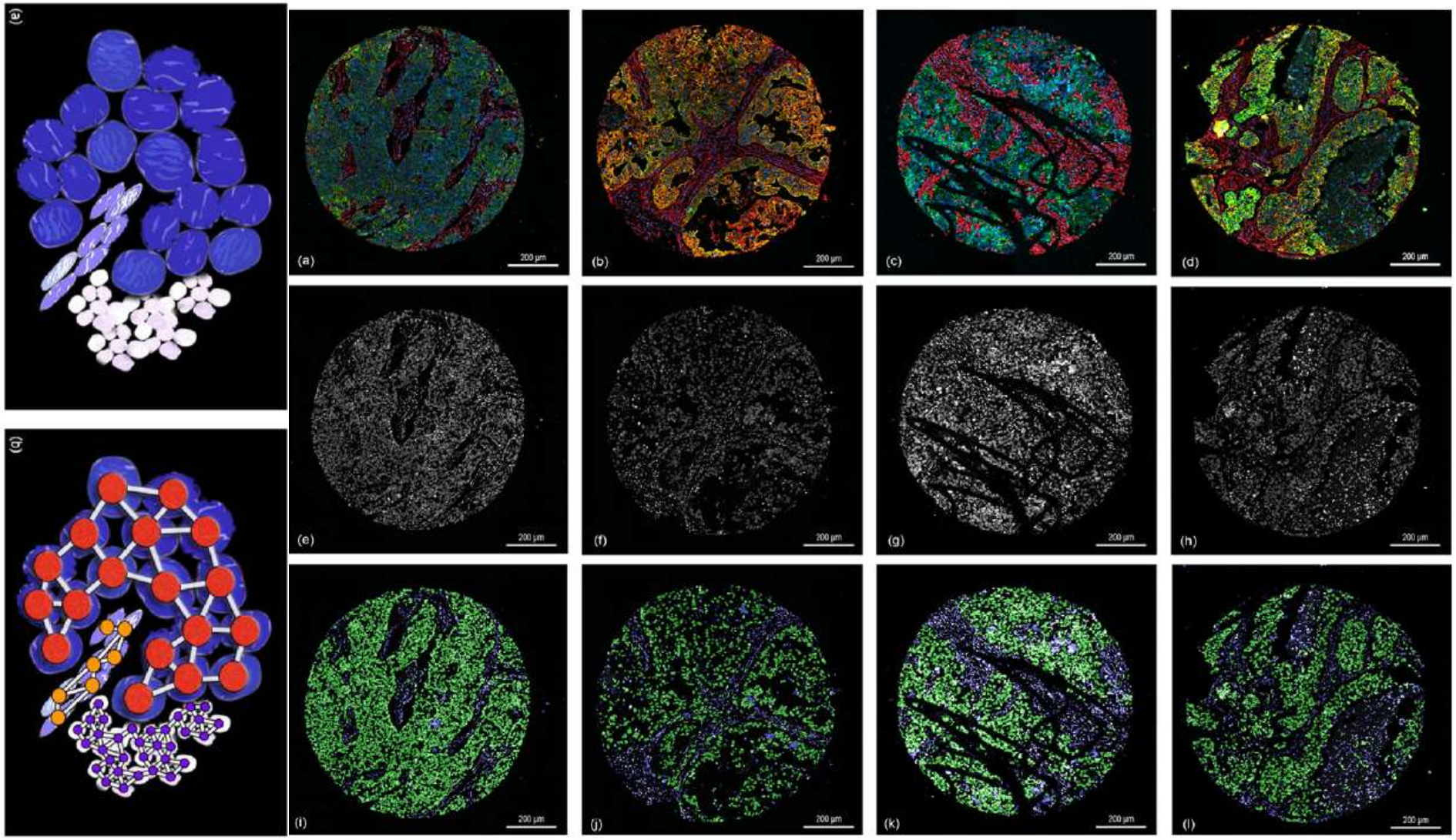




Rimm DL et al. Nature Biotech 2006

Automatic Tumor-Stroma Separation in Fluorescence TMAs Enables the Quantitative High-Throughput Analysis of Multiple Cancer Biomarkers

PLoS ONE 6(12): e28048. doi:10.1371/journal.pone.0028048



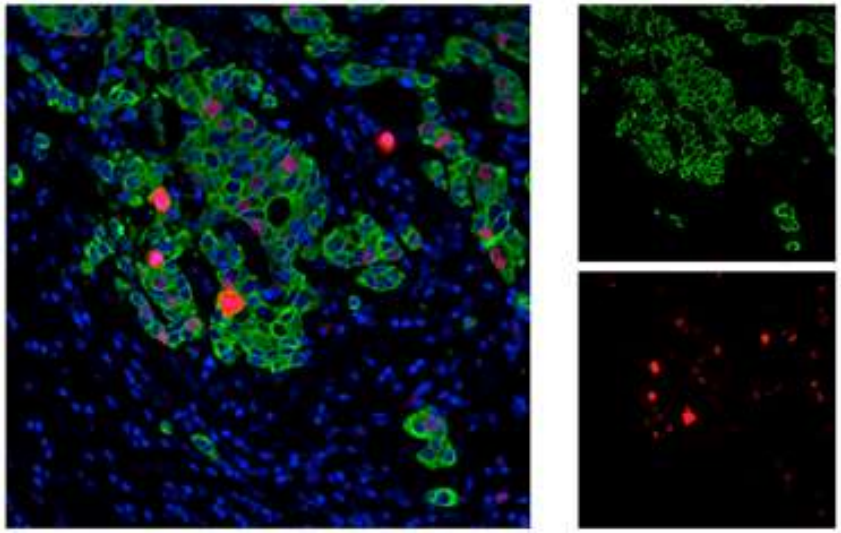
Journal of the National Cancer Institute, Vol. 97, No. 24, December 21, 2005

ARTICLES

Automated Quantitative Analysis (AQUA) of In Situ Protein Expression, Antibody Concentration, and Prognosis

Anthony McCabe, Marisa Dolled-Filhart, Robert L. Camp, David L. Rimm

Assessment of cell proliferation in the tumor epithelial cell compartment using AQUA



Green = CK Red = Ki-67 Blue = Dapi

Journal of the National Cancer Institute, Vol. 97, No. 24, December 21, 2005

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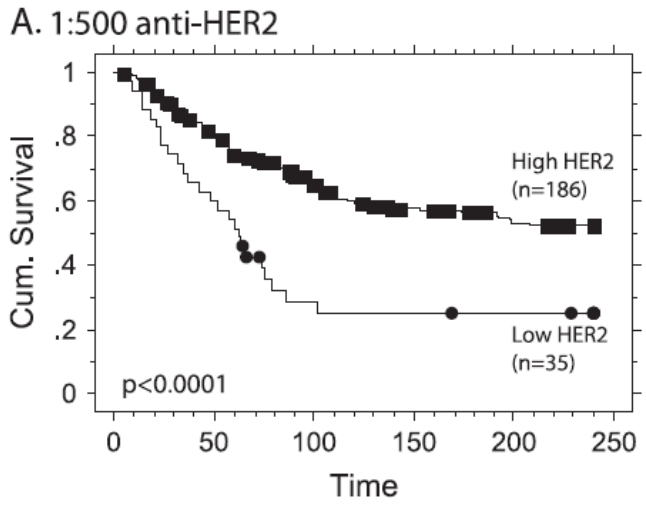
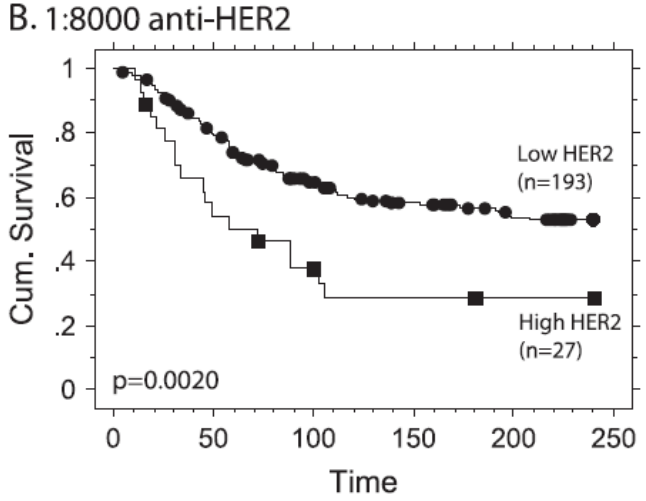
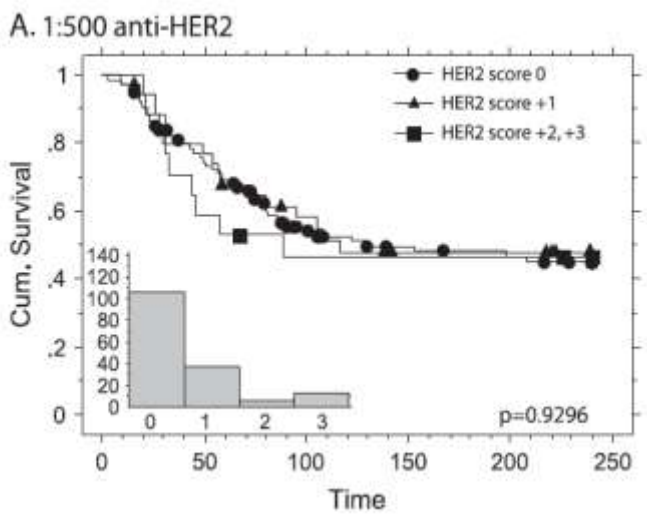
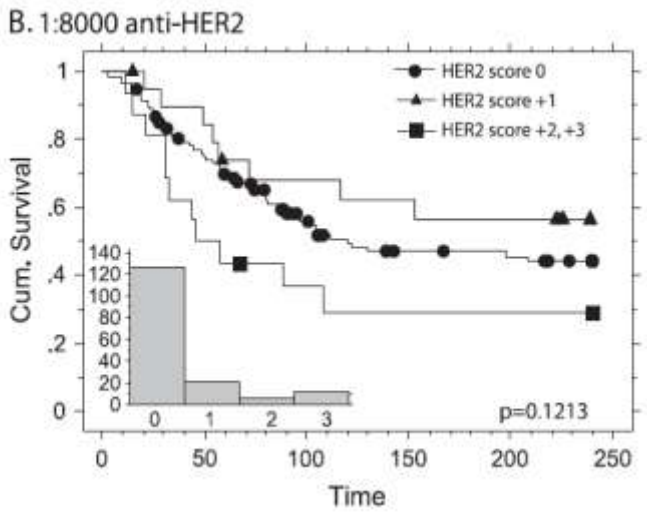
Anthony McCabe, Marisa Dolled-Filhart, Robert L. Camp, David L. Rimm





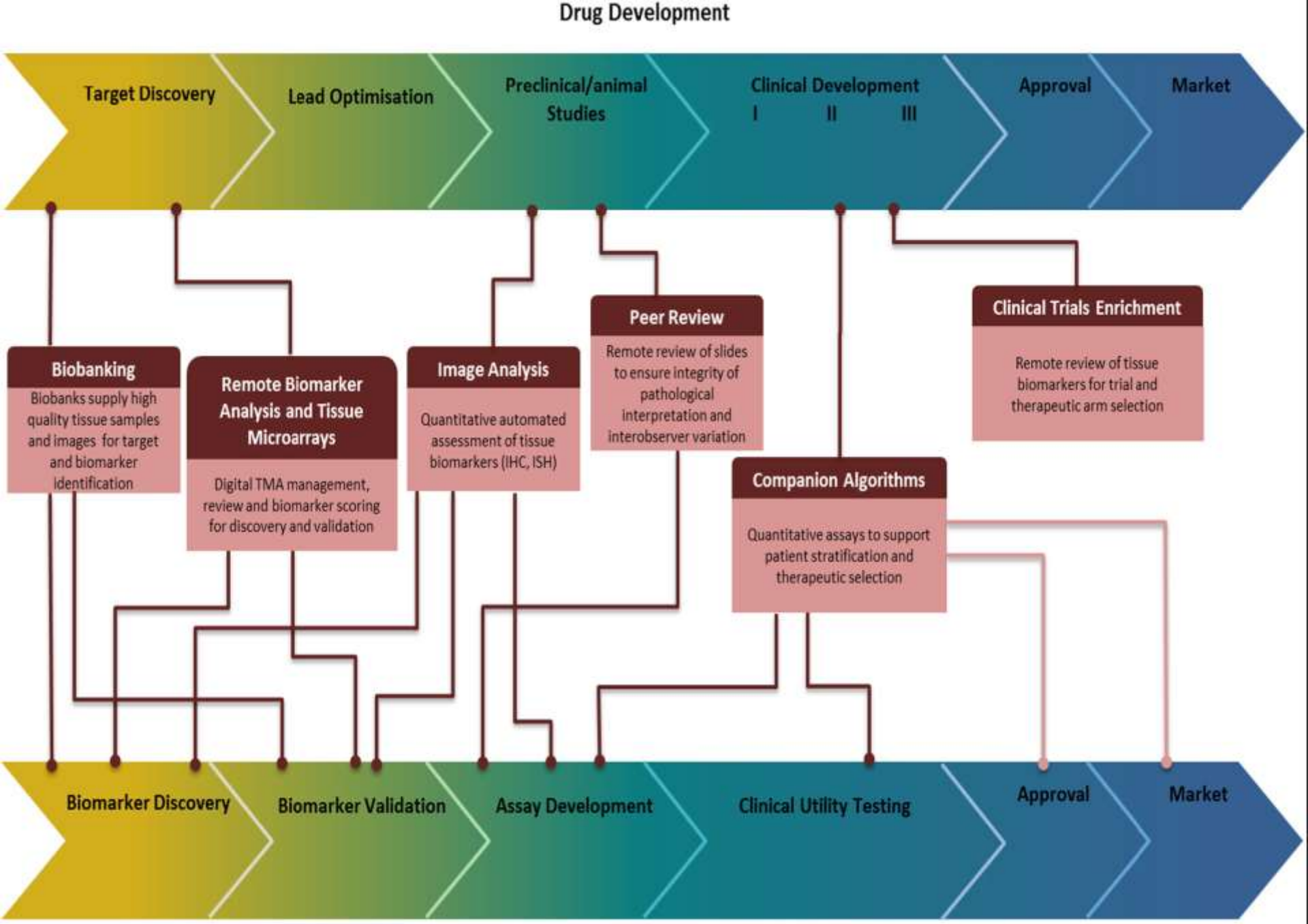




Drug Development

Drug Development

Biomarker Development



Hamilton et al. Methods 2014 (en prensa)

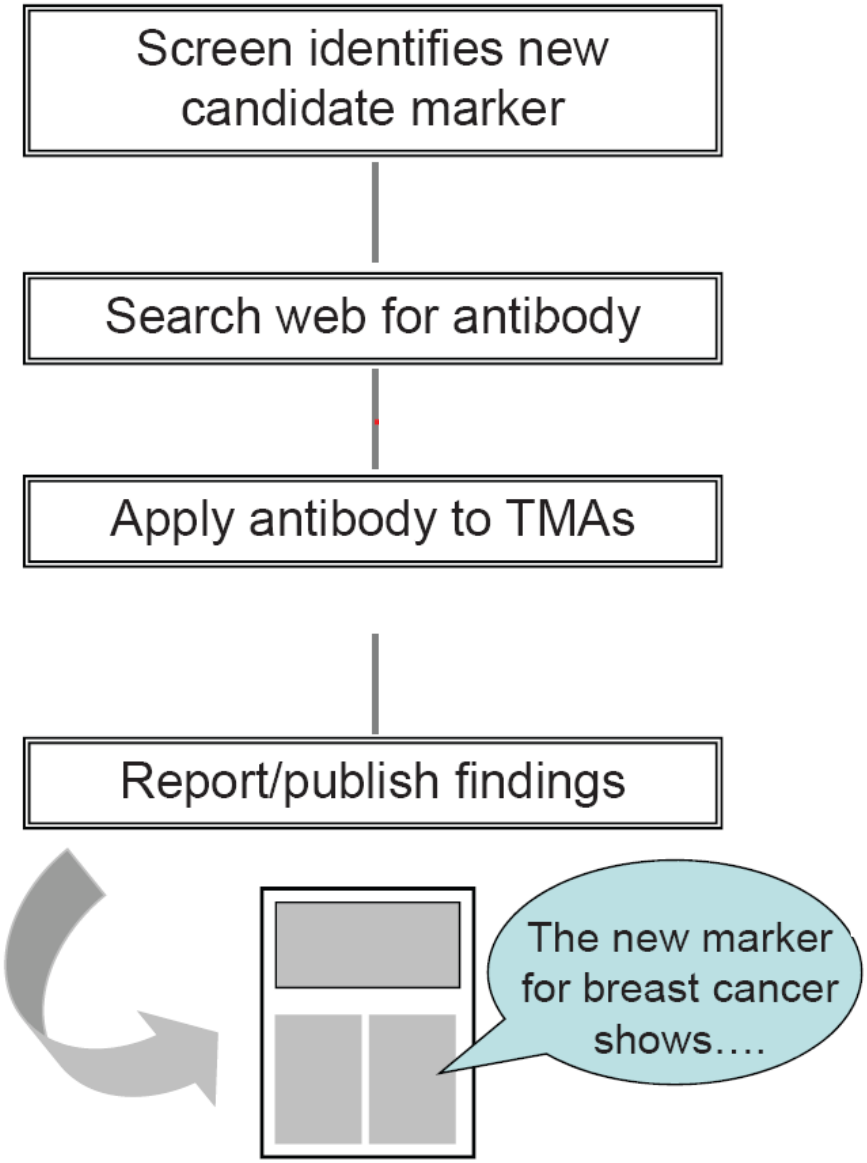


Figure 1 Schematic representation of the validation process of novel biomarkers by using immunohistochemistry.

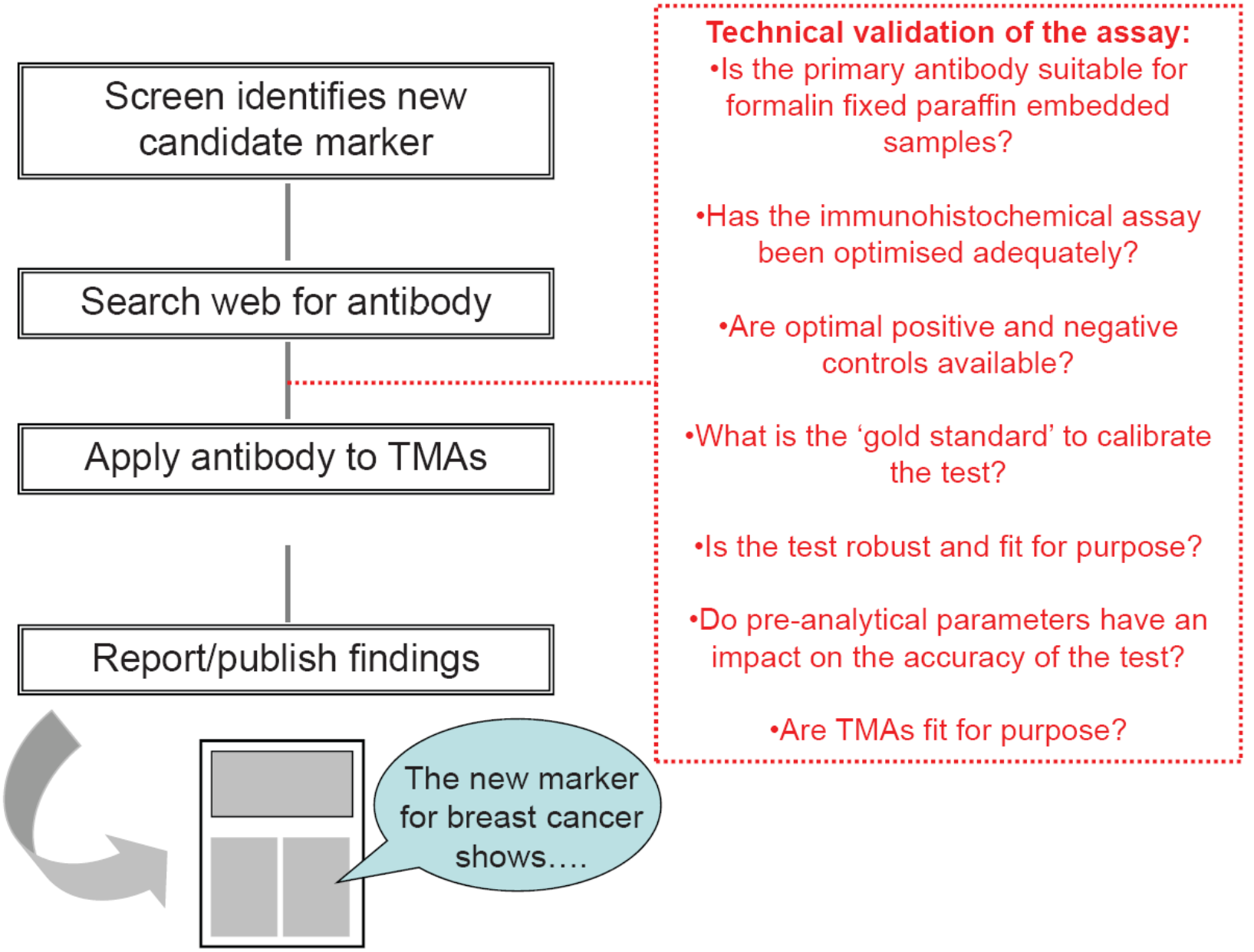


Figure 1 Schematic representation of the validation process of novel biomarkers by using immunohistochemistry.

Table 3. Sources of HER2 Testing Variation

Preanalytic
Time to fixation
Method of tissue processing
Time of fixation
Type of fixation
Analytic
Assay validation
Equipment calibration
Use of standardized laboratory procedures
Training and competency assessment of staff
Type of antigen retrieval
Test reagents
Use of standardized control materials
Use of automated laboratory methods
Postanalytic
Interpretation criteria
Use of image analysis
Reporting elements
Quality assurance procedures
Laboratory accreditation
Proficiency testing
Pathologist competency assessment

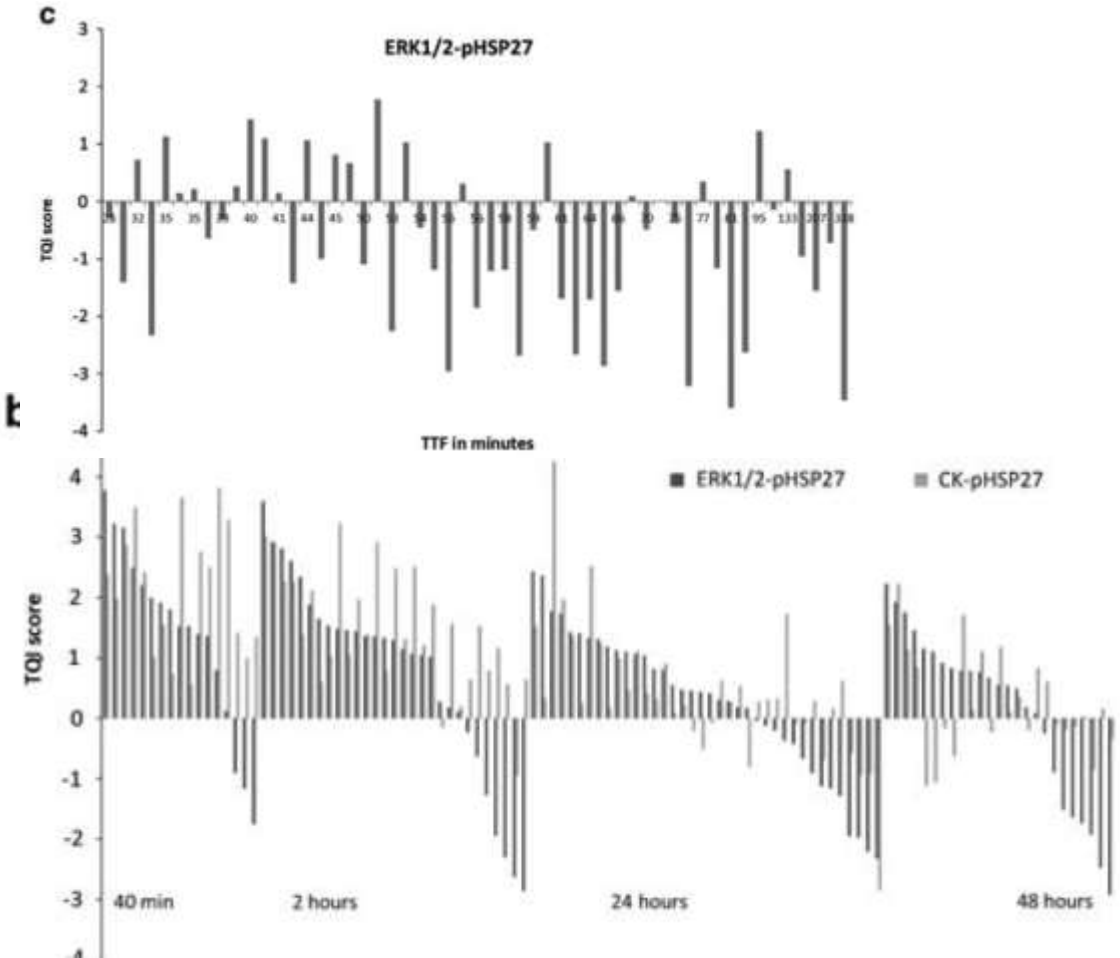
Abbreviation: HER2, human epidermal growth factor receptor 2.

Wolff et al, JCO 2007

A tissue quality index: an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue

Laboratory Investigation (2014) **94,** 467–474;

Veronique M Neumeister[1], Fabio Parisi[1], Allison M England[1], Summar Siddiqui[1], Valsamo Anagnostou[1], Elizabeth Zarrella[1], Maria Vassilakopolou[1], Yalai Bai[1], Sasha Saylor[1], Anna Sapino[2], Yuval Kluger[1,2], David G Hicks[3], Gianni Bussolati[2], Stephanie Kwei[4] and David L Rimm[1]



Calidad

- **Garantía de calidad de inmunohistoquímica (IHQ) y patología molecular (PM)**
  - Módulo de patología molecular
  - Acceso al Programa de Calidad de IHQ y P. Molecular
- Inscripción IHQ
- Garantía de calidad de diagnóstico
- Inscripción GCD
- Programa de Calidad ValidaRAS
- Garantía de Calidad CONFIRMA HER2
  - Preguntas Frecuentes CONFIRMA HER2
  - Programa de Puntos CONFIRMA HER2
- Inscripción CONFIRMA HER2
- Informes de rondas

El Programa para la Garantía de Calidad en Patología (GCP) de la SEAP-IAP

Área privada para socios de la SEAP-IAP

El programa de calidad de la SEAP comenzó en 2004. Desde entonces estamos aprendiendo y adquiriendo experiencia para alcanzar los objetivos básicos: cumplir con los calendarios y dar la información necesaria a los laboratorios para mejorar la calidad de su diagnóstico. Hoy día somos todos conscientes de que los Sistemas Externos de Garantía de Calidad son necesarios en Patología como antes lo creyeron otros especialistas básicos como hematólogos, bioquímicos y microbiólogos.

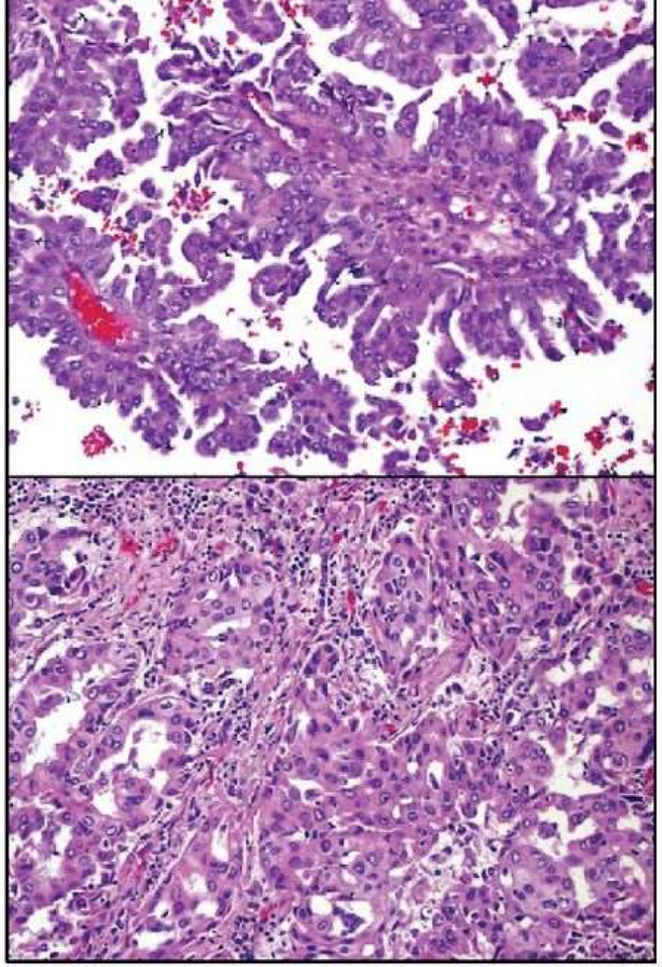
Como teníamos previsto desde el principio, hemos ido ampliando el espectro del control en Patología y por ello, actualmente el programa consta de los siguientes módulos:

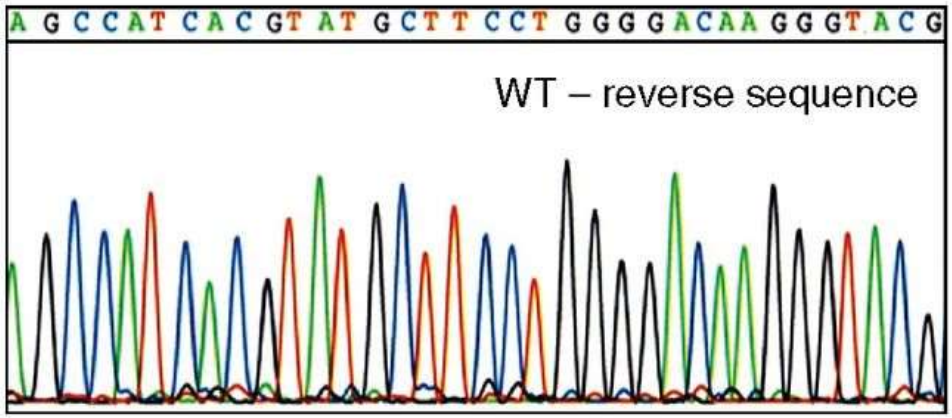
- Sección garantía de calidad de inmunohistoquímica (IHQ) y patología molecular (PM)
  - Módulo de Patología Quirúrgica
  - Módulo de HER2-neu
  - Módulo de mama
  - Módulo de tejido linfoide
  - Módulo de patología molecular

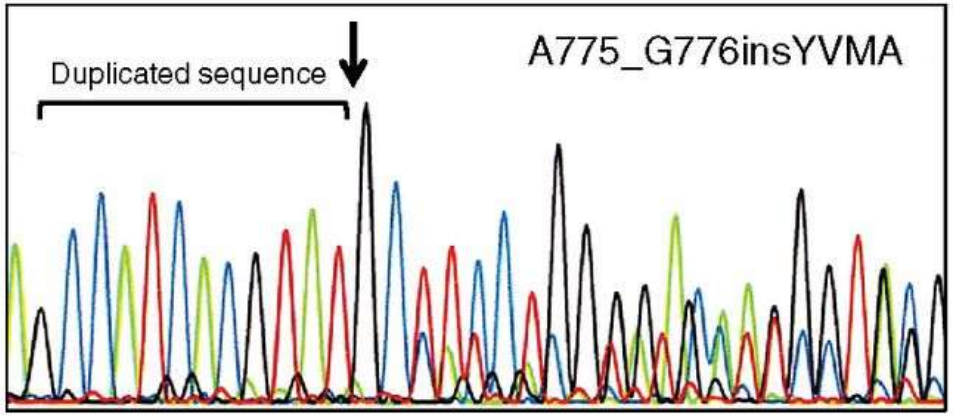
- Sección garantía de calidad de diagnóstico
  - Módulo de Diagnóstico en Citopatología
  - Módulo de Diagnóstico en Patología Quirúrgica

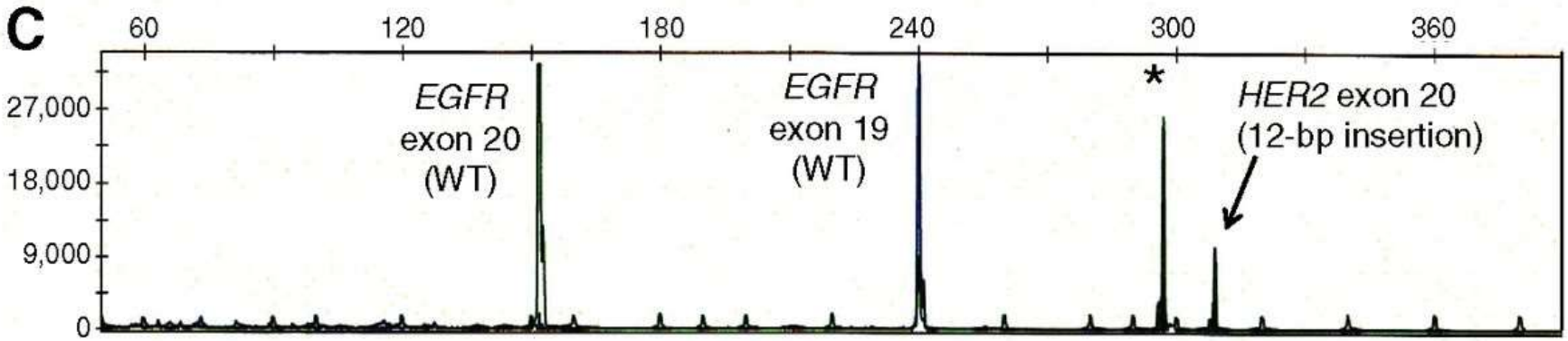
A la hora de seleccionar unos u otros programas es importante señalar que los hay personales (Diagnóstico) e institucionales (IHQ y PM). En este último caso se precisa del nombre de una persona de contacto que represente al laboratorio, servicio o departamento. Para un adecuado funcionamiento es imprescindible recibir las inscripciones antes del 31 de diciembre del año en curso.

Programa de Calidad ValidaRAS



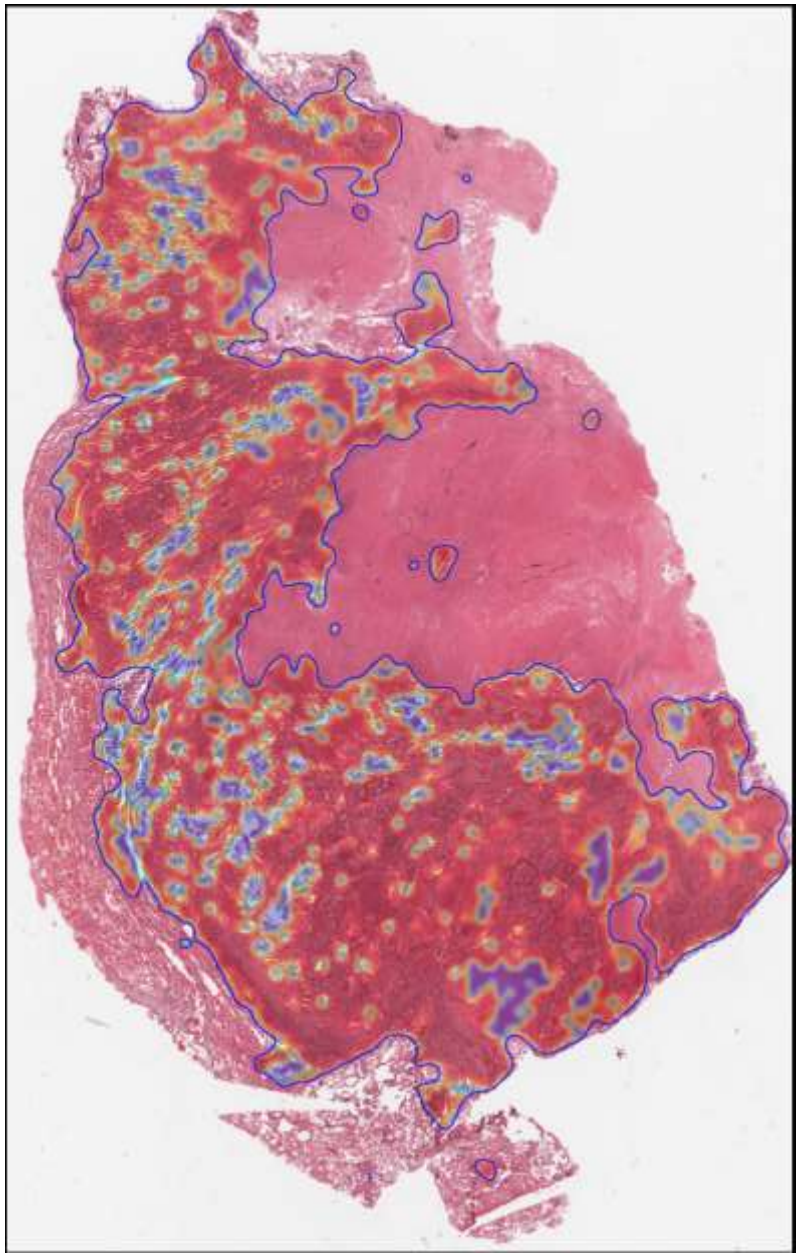

A G C C A T C A C G T A T G C T T C C T G G G G A C A A G G G T A C G
WT – reverse sequence


Duplicated sequence
A775_G776insYVMA

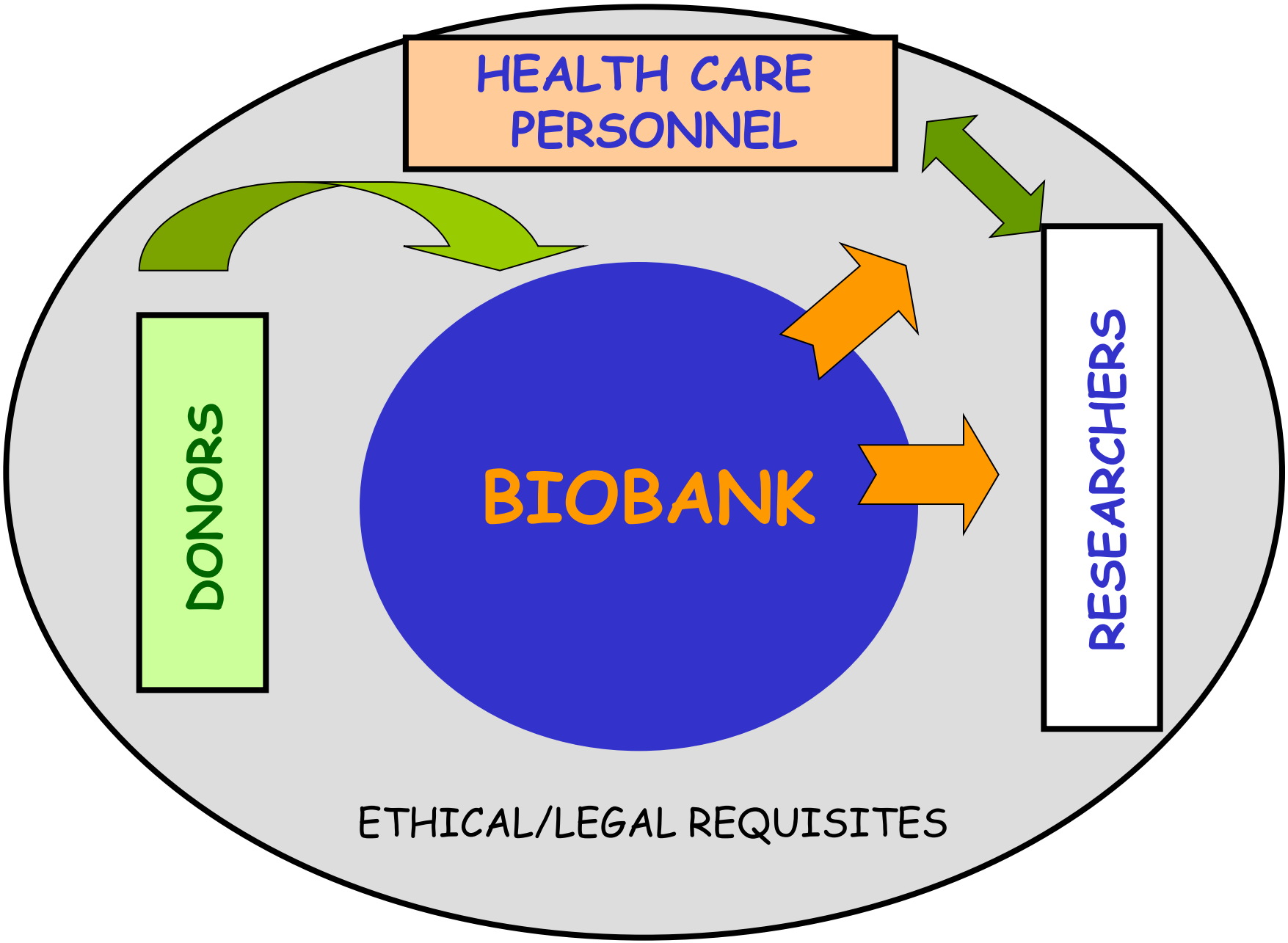

C
60
120
180
240
300
360
27,000
18,000
9,000
0
EGFR exon 20 (WT)
EGFR exon 19 (WT)
*
HER2 exon 20 (12-bp insertion)

Dos aplicaciones en marcadores más ‘moleculares’

- Porcentaje de tumor respecto al total de la muestra
- Porcentaje de células tumorales respecto al total de tumor

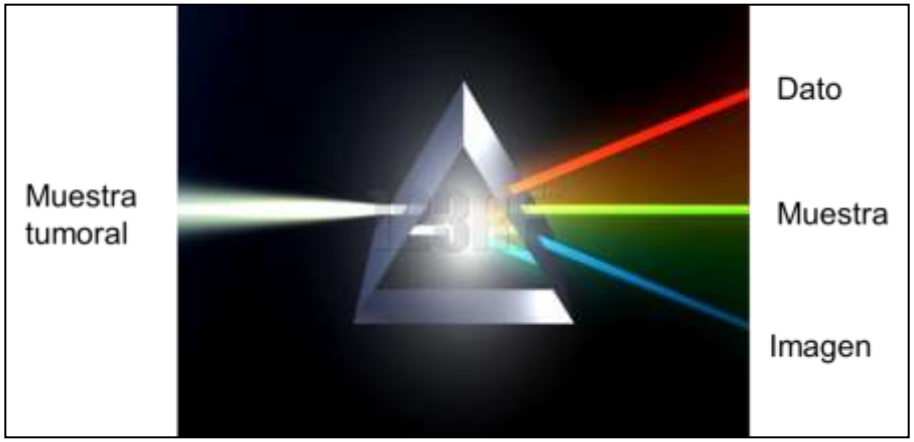


www.pathxl.com

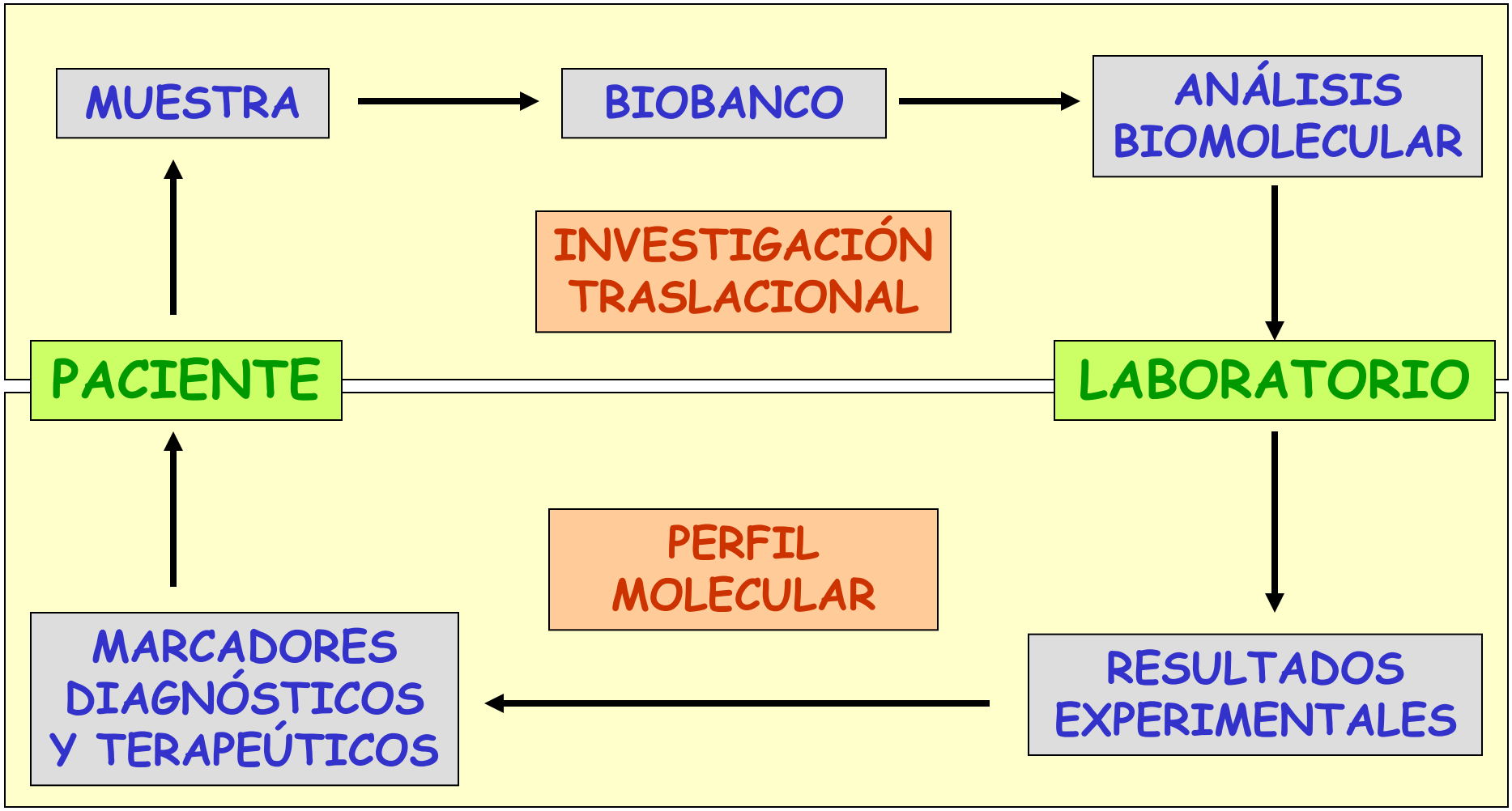

HEALTH CARE PERSONNEL
DONORS
BIOBANK
RESEARCHERS
ETHICAL/LEGAL REQUISITES

Patología digital y biobancos

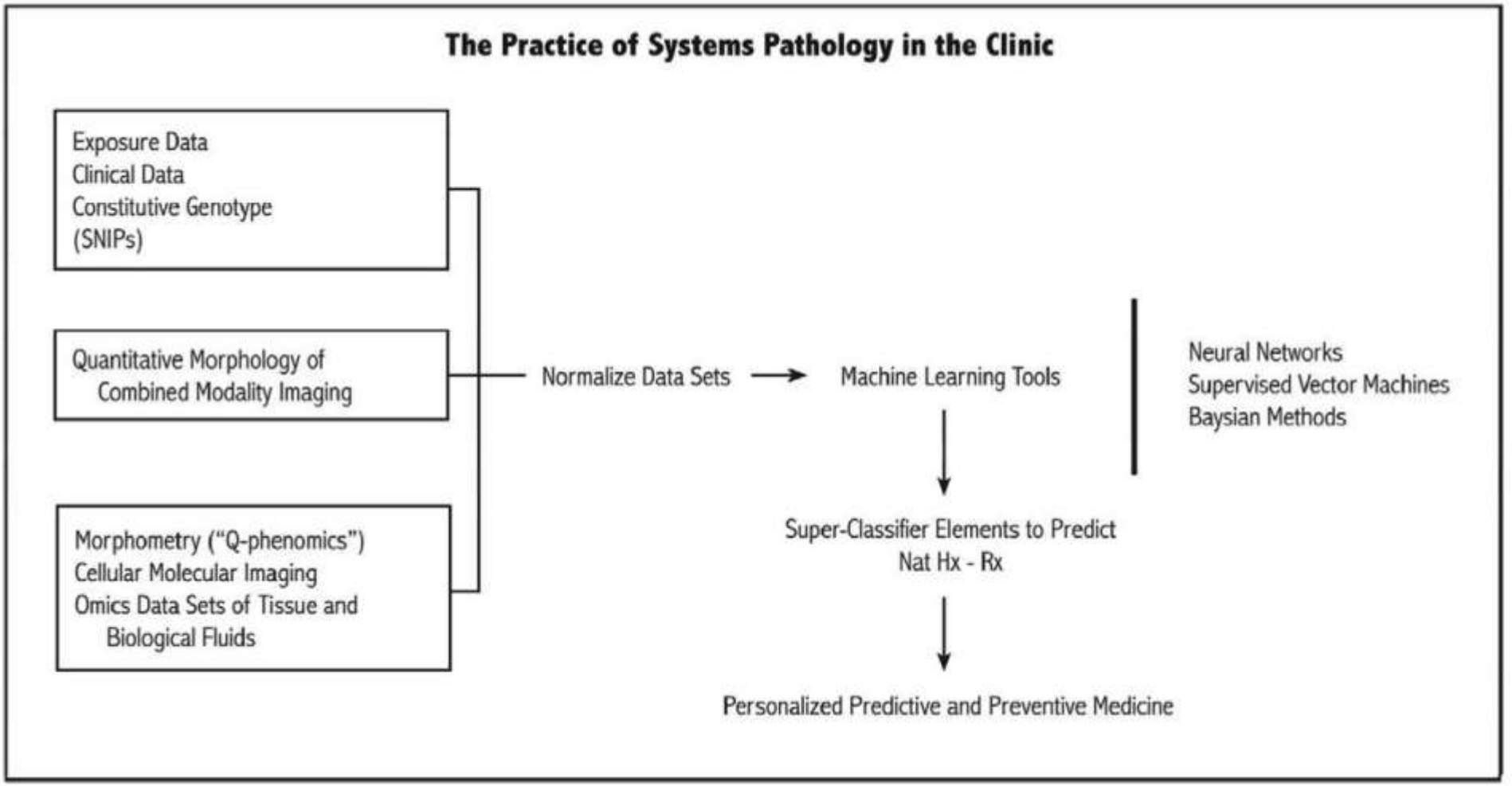
- Archivar preparaciones digitales asociadas a las muestras archivadas físicamente.
- Revisar ágilmente las imágenes digitales de manera previa a una provisión de muestras.
- Enriquecer la información asociada a las muestras (clínica, molecular, etc.) mediante el análisis de biomarcadores.



El biobanco, en el centro de la medicina personalizada

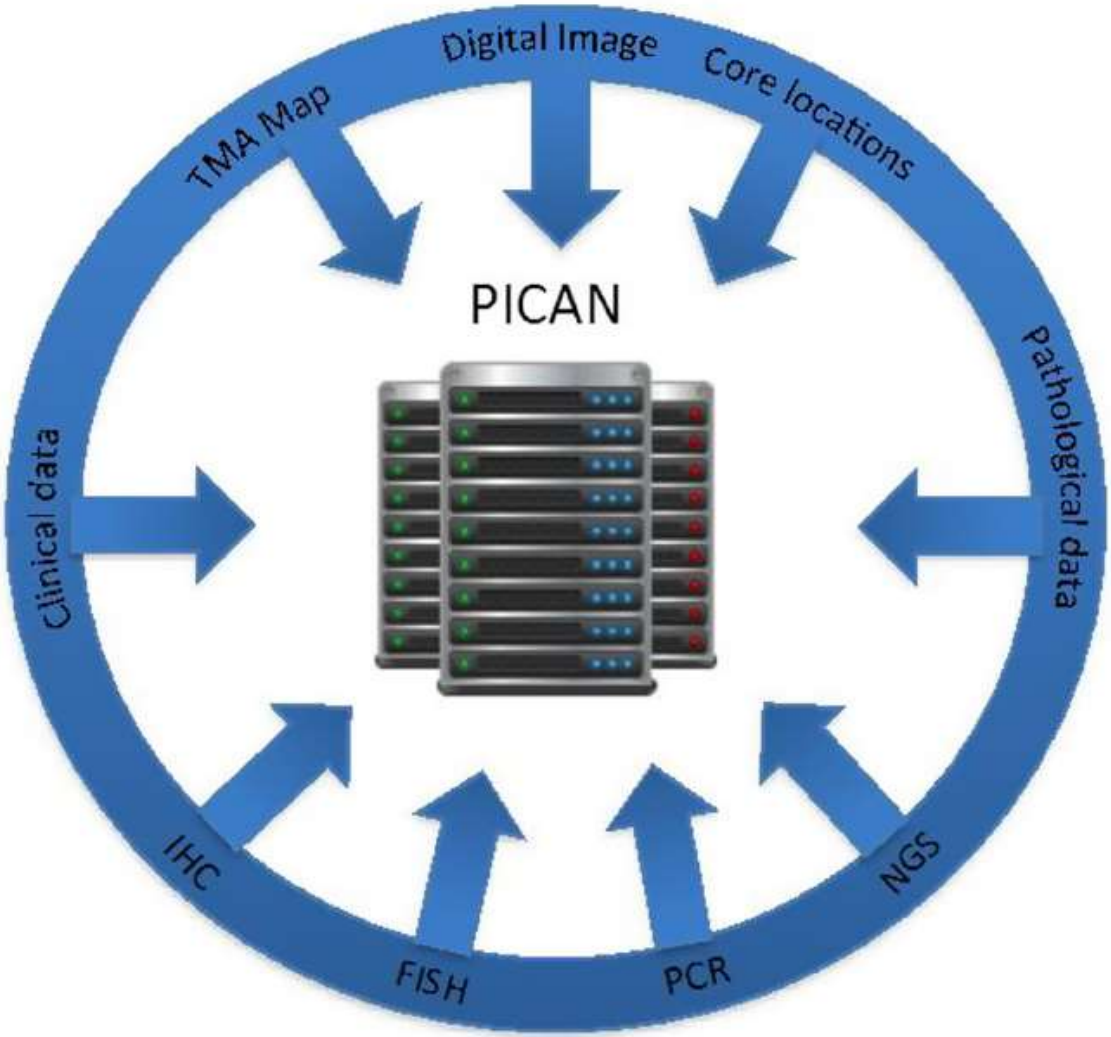


¿Medicina de sistemas?



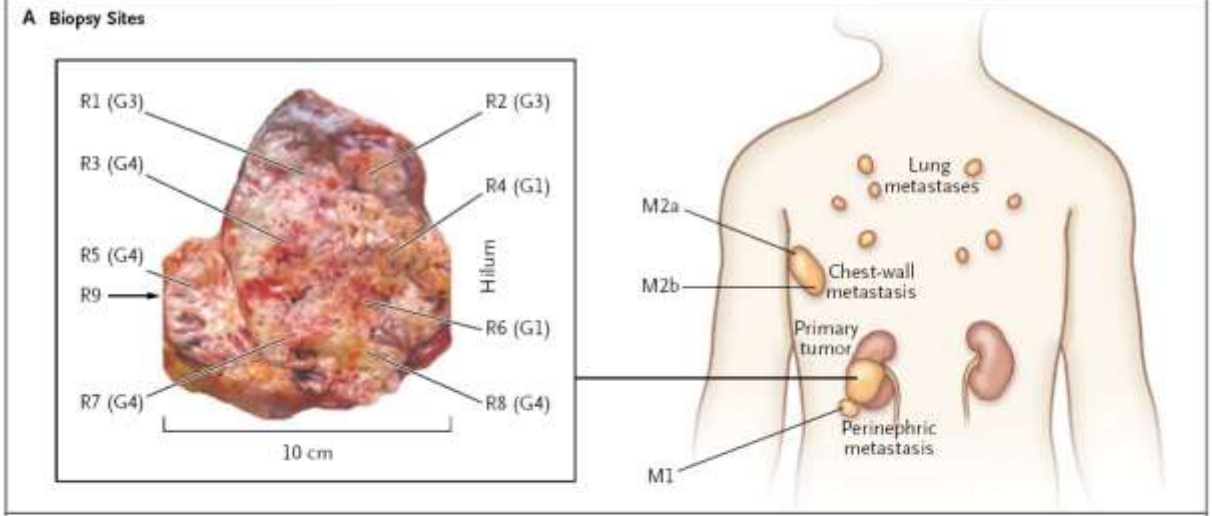
Costa J. Arch Pathol Lab Med 2008

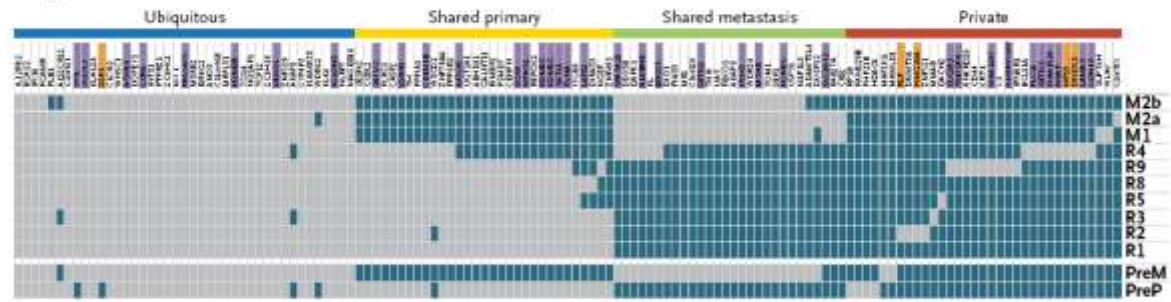
“Integrómica”

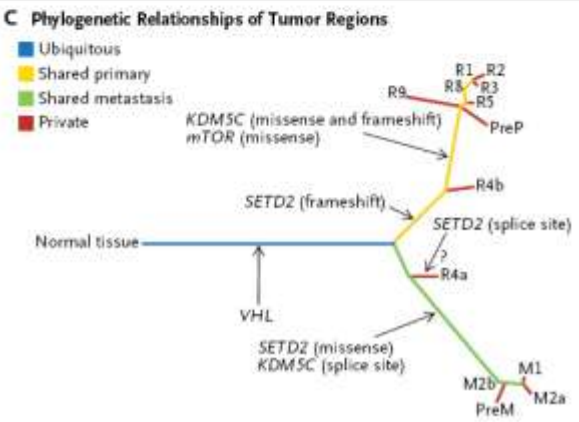


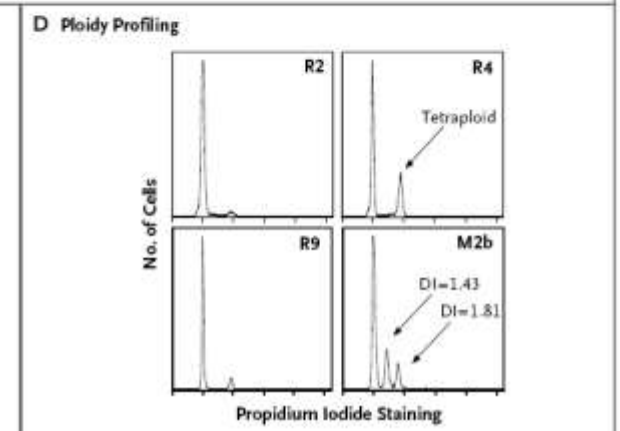
http://conference.ncri.org.uk/abstracts/2013/abstracts/B12.htm

N Engl J Med 2012;366:883-92.









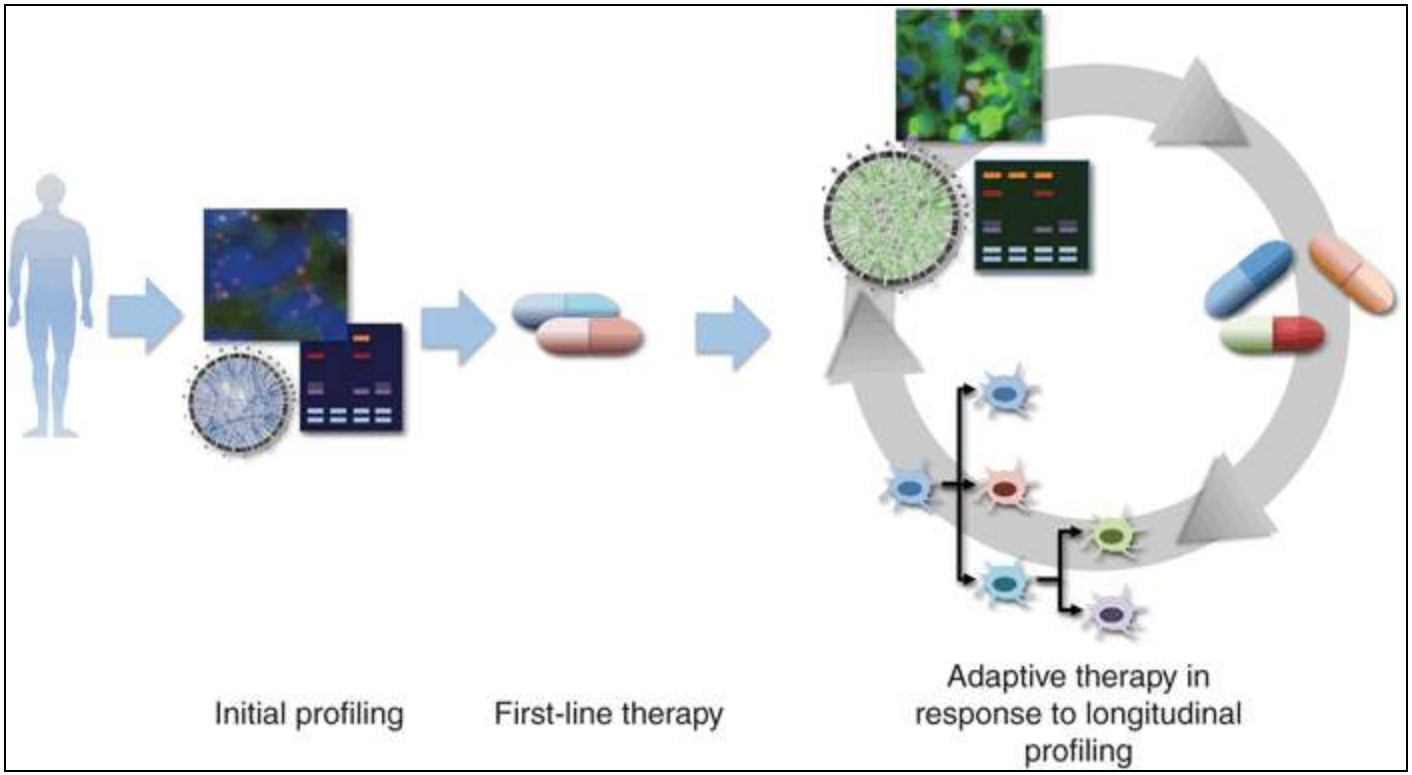
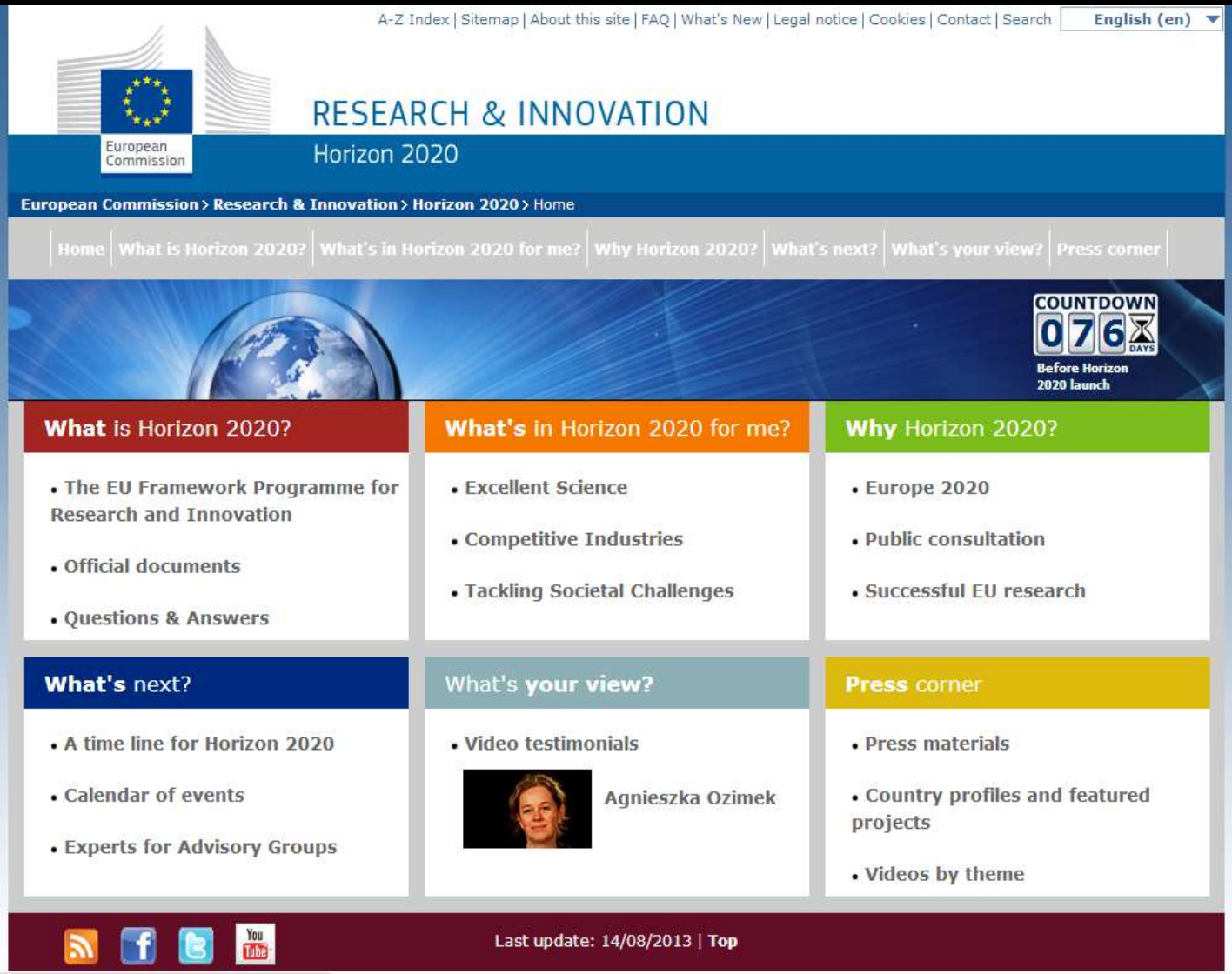


Figure 3 Envisaging the future of personalized precision medicine for cancer treatment. Future approaches will be based on adaptive therapy in response to information from tumor profiling using multiple technologies, including next-generation sequencing to identify predictive and resistance biomarkers, and incorporating analyses of clonal, morphological, and anatomical heterogeneity and their variations longitudinally in real time.

Horizon 2020



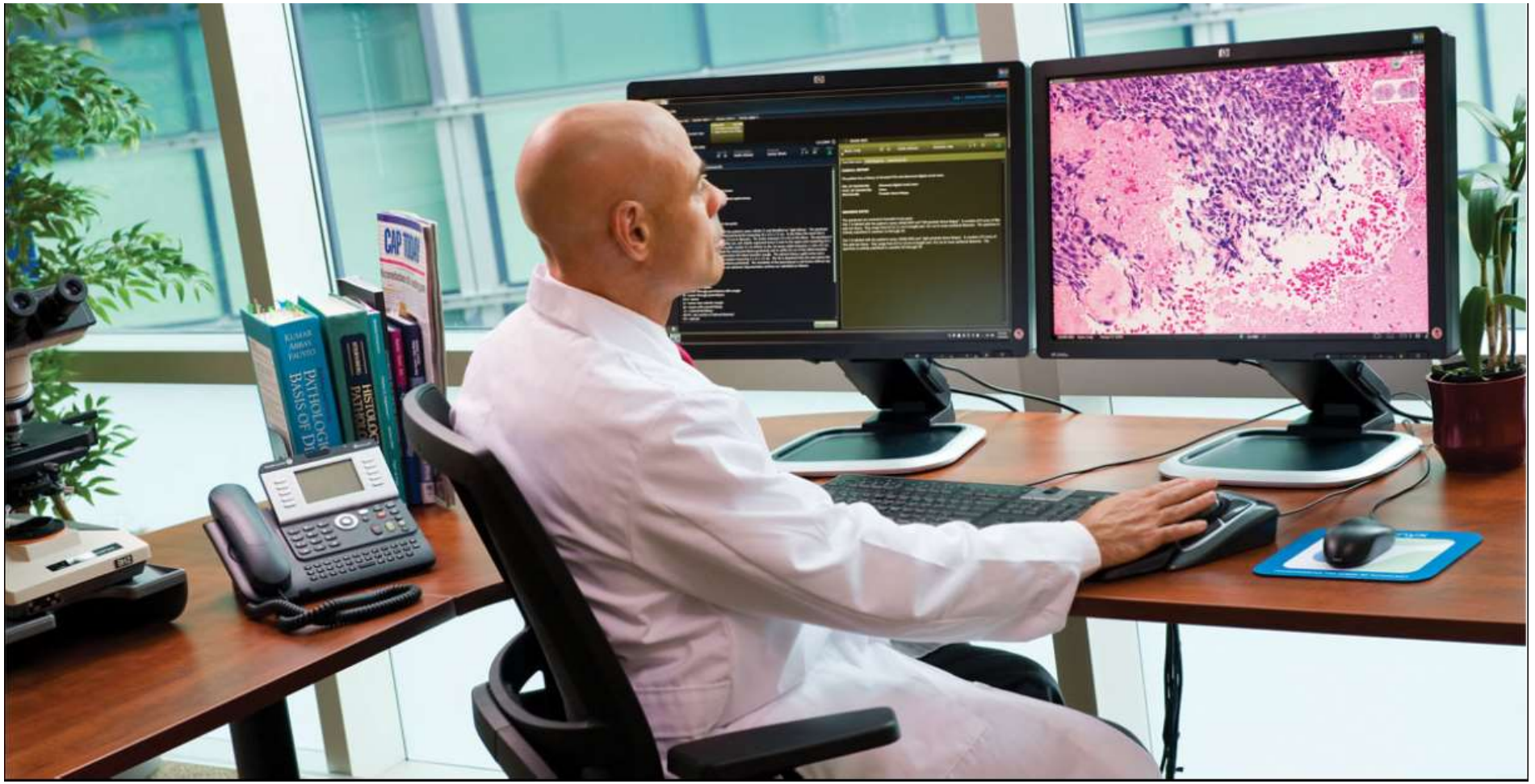
PHC 31 – 2015: Digital representation of health data to improve disease diagnosis and treatment

Work will propose new decision support systems based on a complex integration of heterogeneous data sources and subject-specific computer models. This will enable an integrated data analysis, and will present a highly visual data representation, using userfriendly interactive exploratory interfaces in order to assure usability and acceptability.

ethical considerations. The models should be already available, multi-level and multi-scale and will be integrated with the individual and population data relevant for the targeted clinical situations, e.g. the required molecular and cellular data, including genomics and epigenomics, in vivo and in vitro imaging data, or data on administration of therapeutics and on nutrition/exposure to environmental factors and will be linked when relevant with computer models of personalised physiology, functional disorders and other diseases. The proposed systems should take advantage of the personal

Guión

- Un entorno cambiante
- Necesitamos mirar de manera diferente.
- Algunos retos.
  - La IHQ y patología molecular morfológica
  - Medir la heterogeneidad
  - Desarrollando biomarcadores
  - Gigantes con pies de barro
  - La patología molecular no morfológica
- Hacia una visión global


CAP TODAY

¡Gracias!

enrique.alava.sspa@juntadeandalucia.es