SAMPLE COLLECTION AND PROCESSING
A Risk Management Approach

IMPAKT 2014

Roberto Salgado

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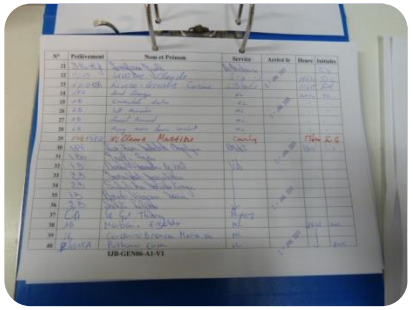
Disclosures

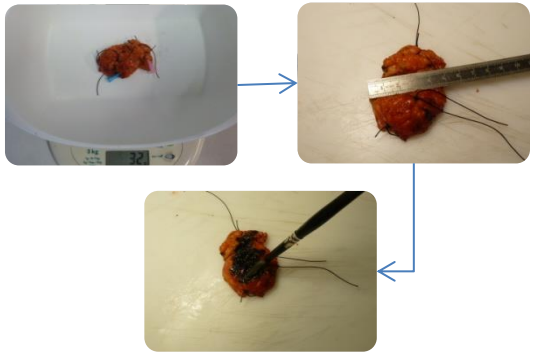
- Advisory relationship Roche
- Advisory relationship Amgen
- Advisory relationship Histogenex

The sample flow in a Pathology Laboratory

MACROSCOPY



RECEPTION → LABELING → REGISTRATION



TISSUE DESCRIPTION → TISSUE HOLDER → VIP

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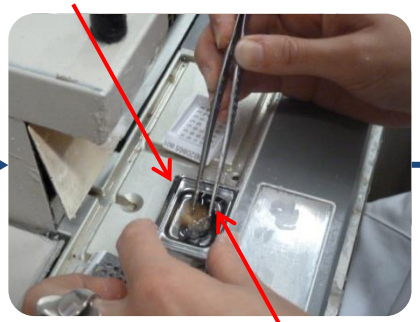
INCLUSION IN PARRAFIN



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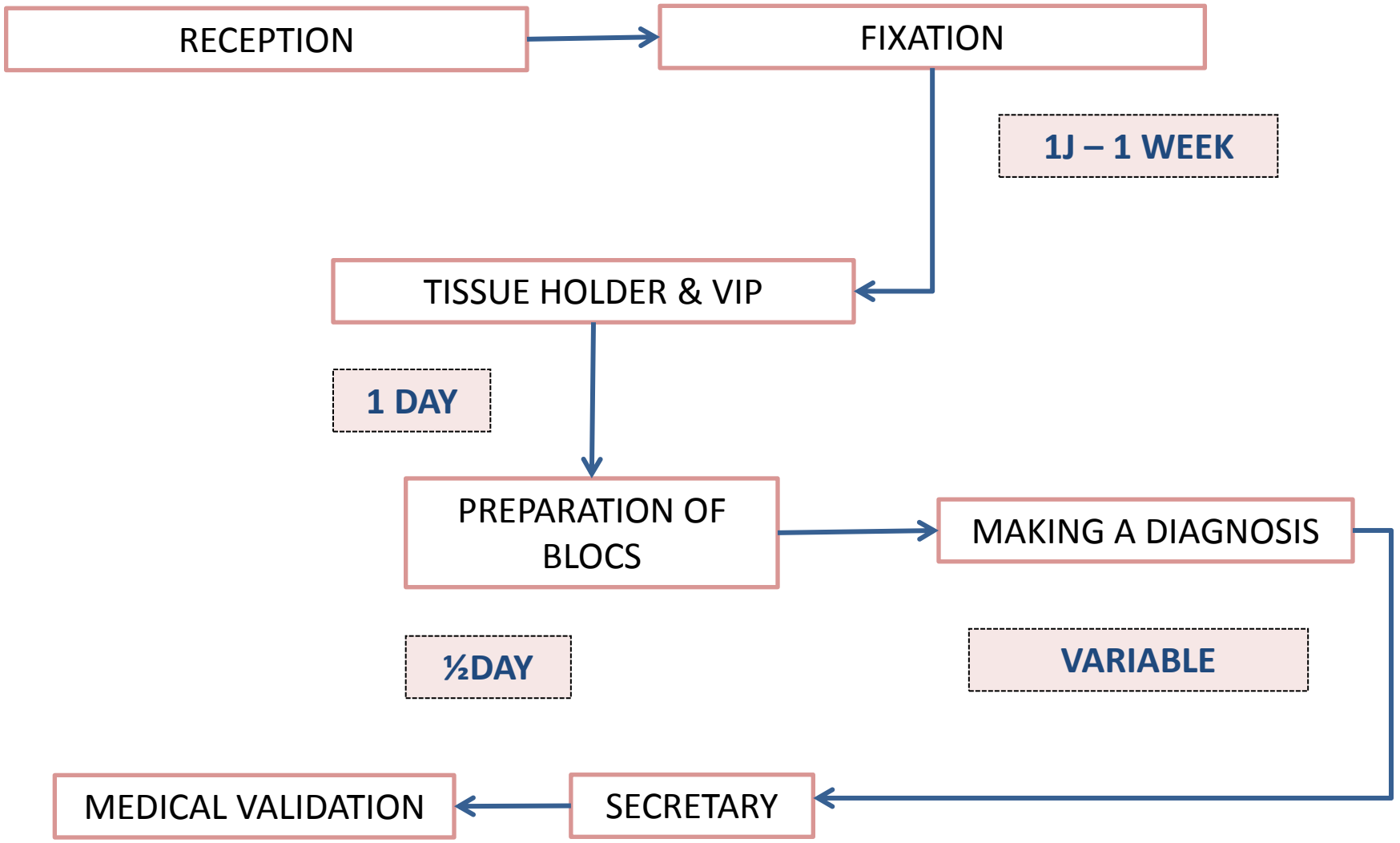
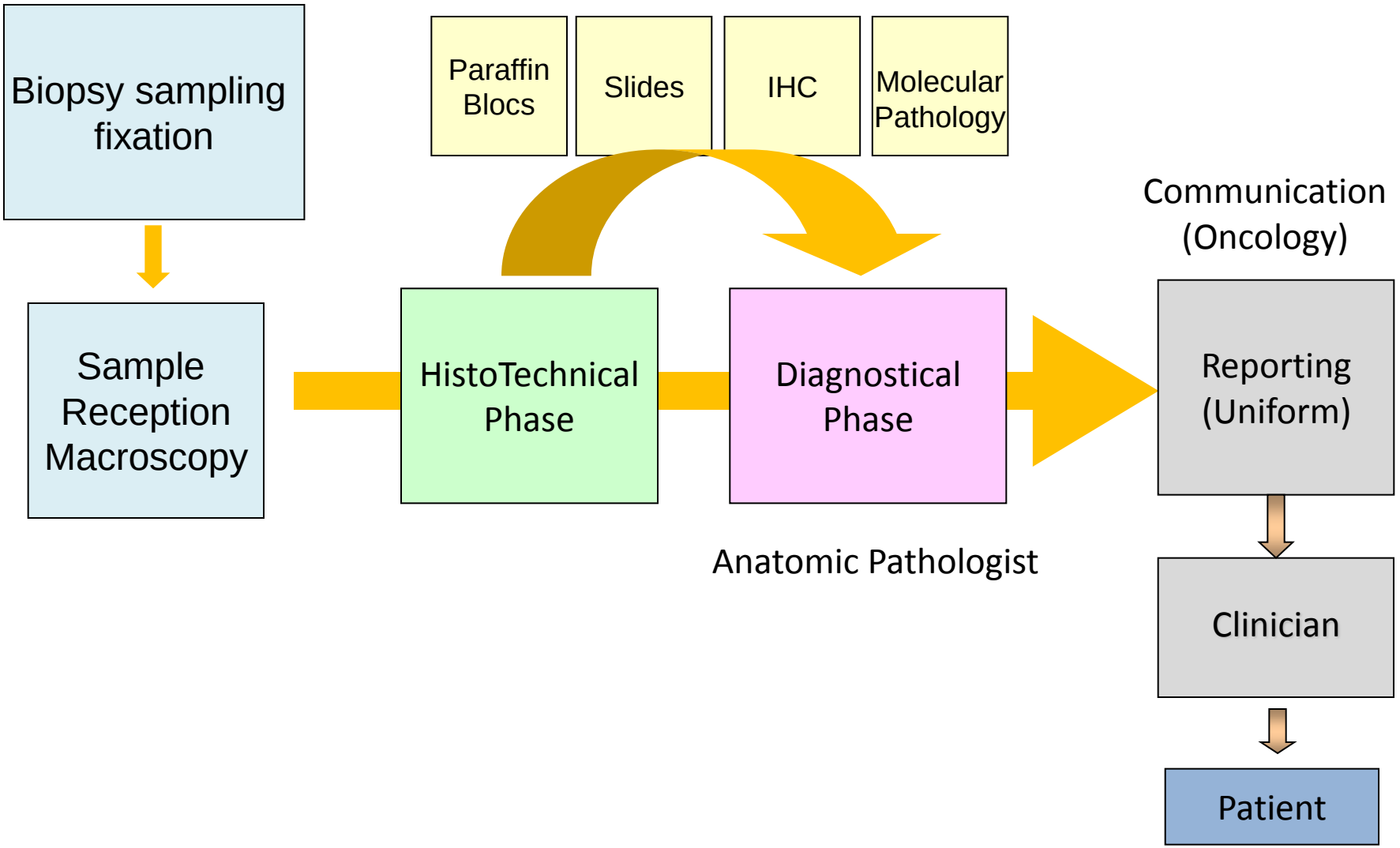
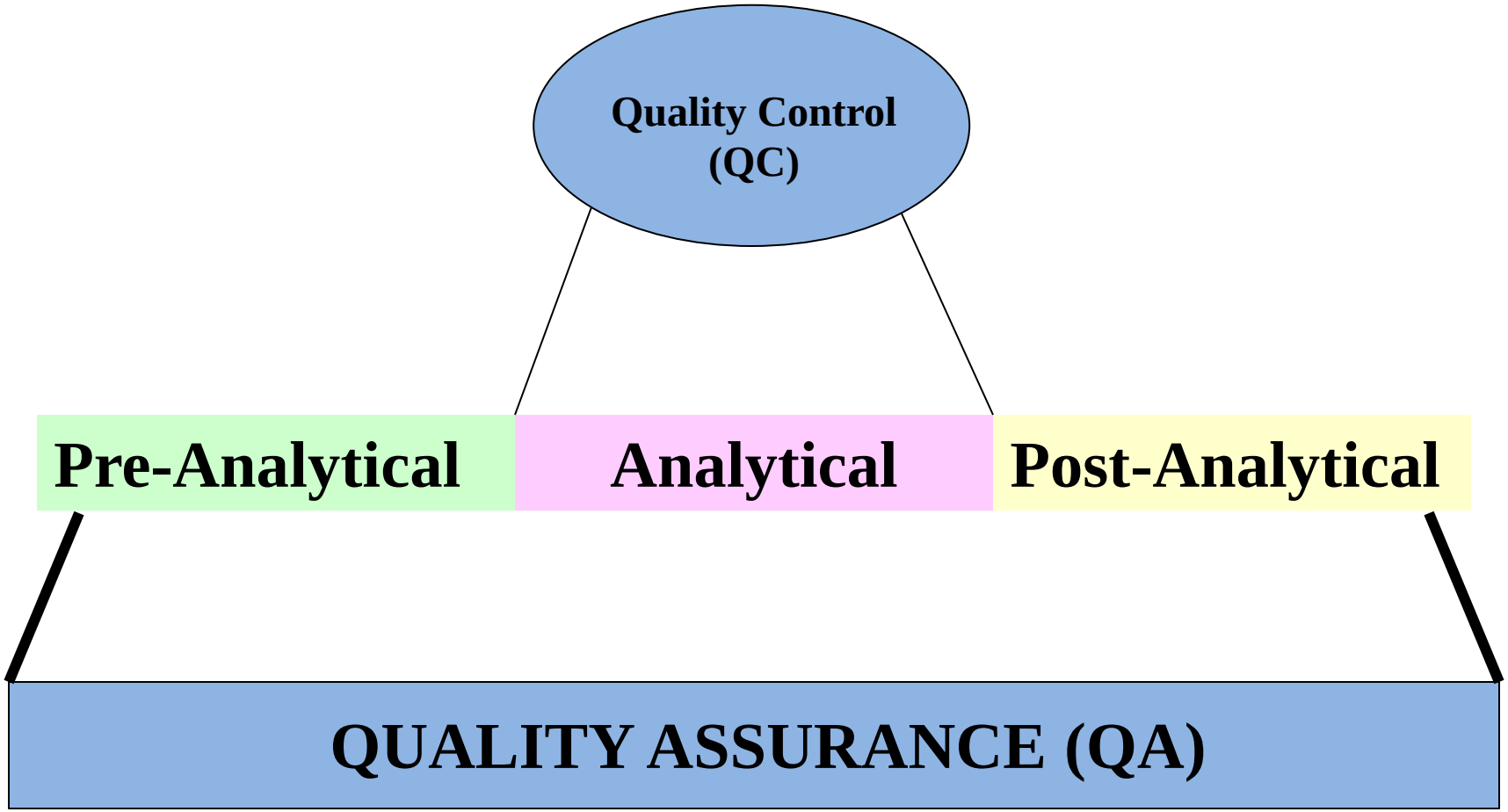
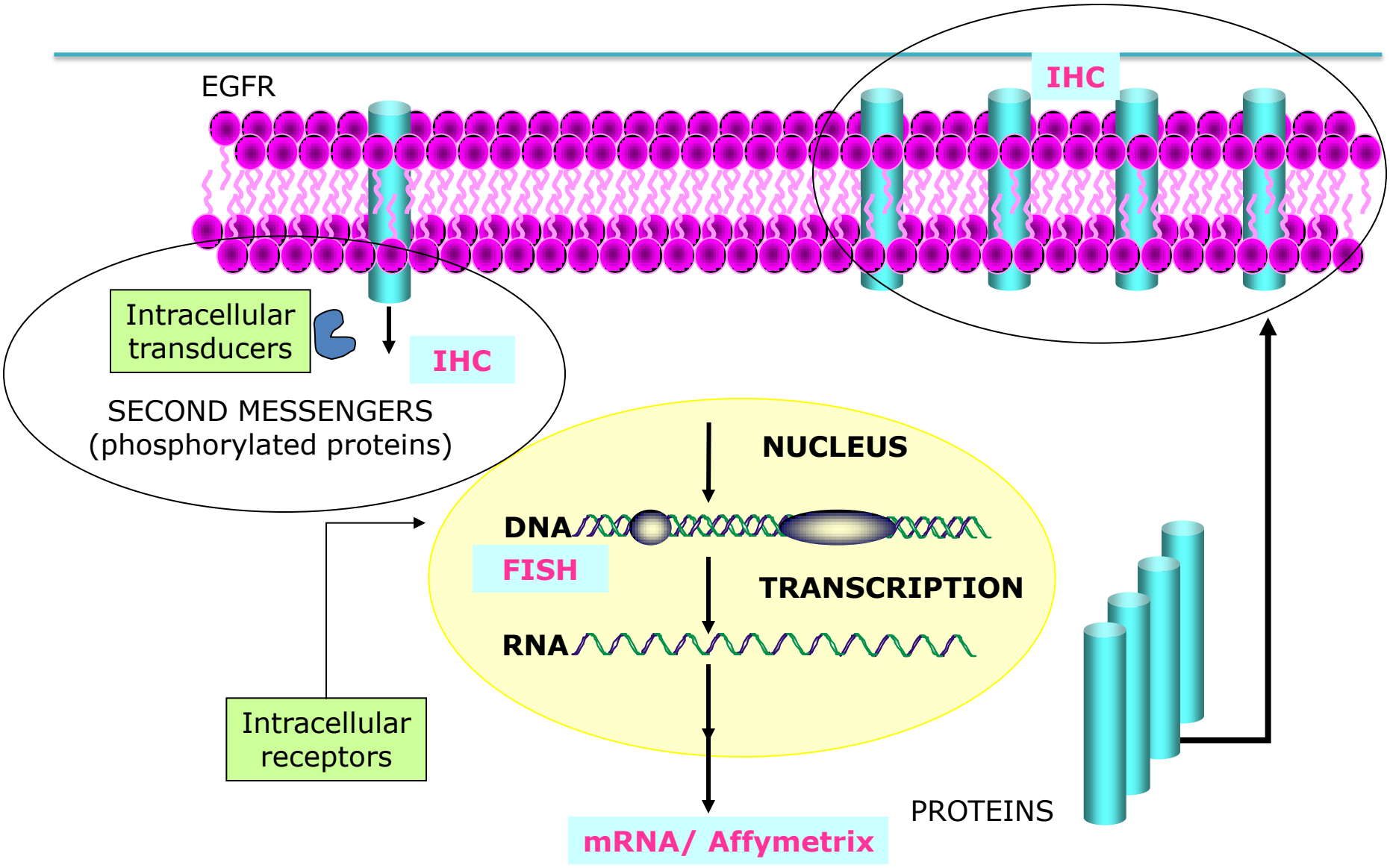
FROM SAMPLE RECEPTION TO DIAGNOSIS



THE PATHOLOGY WORK FLOW




Quality Control
(QC)
Pre-Analytical
Analytical
Post-Analytical
QUALITY ASSURANCE (QA)


EGFR
IHC
Intracellular transducers
IHC
SECOND MESSENGERS (phosphorylated proteins)
NUCLEUS
DNA
FISH
TRANSCRIPTION
RNA
Intracellular receptors
PROTEINS
mRNA/ Affymetrix

Take Home message 1

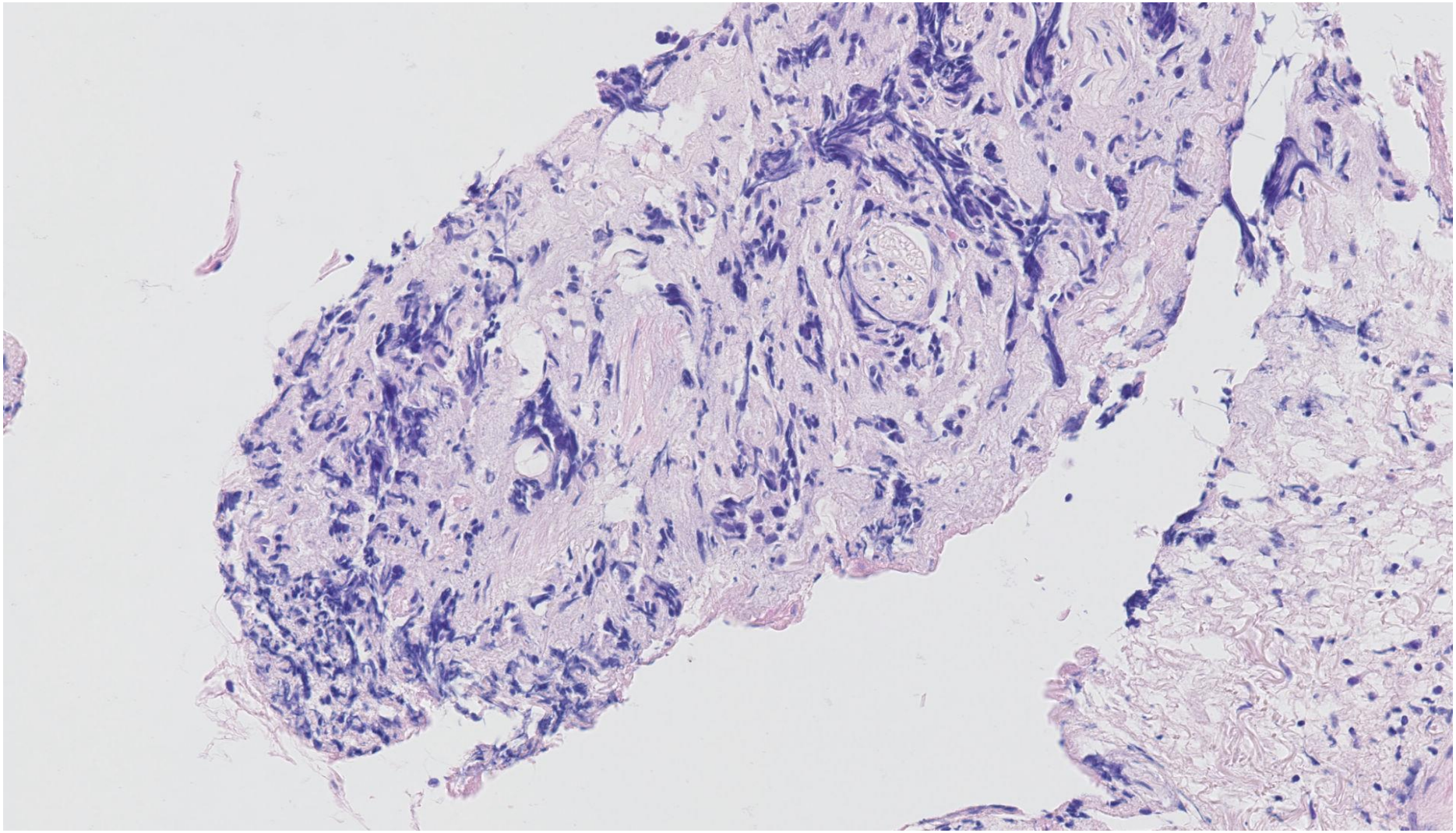
Good Pathology Practices can be **costly** and **time-consuming**

Why is good sample handling important?

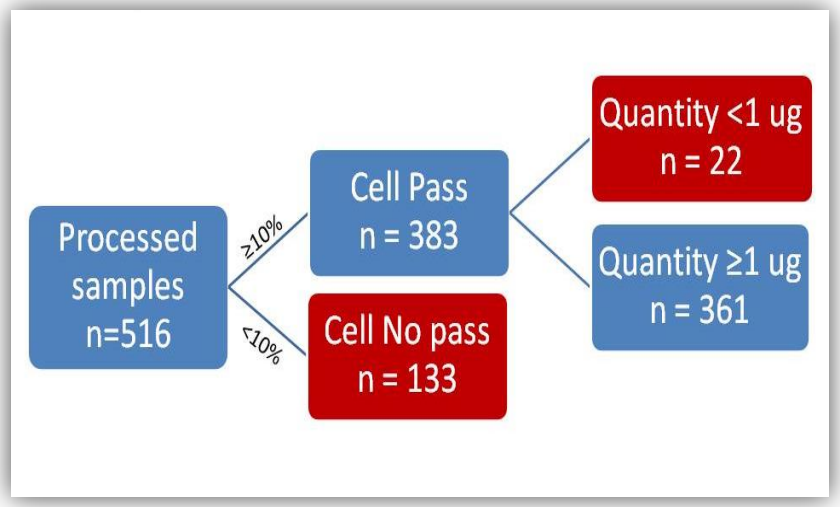
A few examples

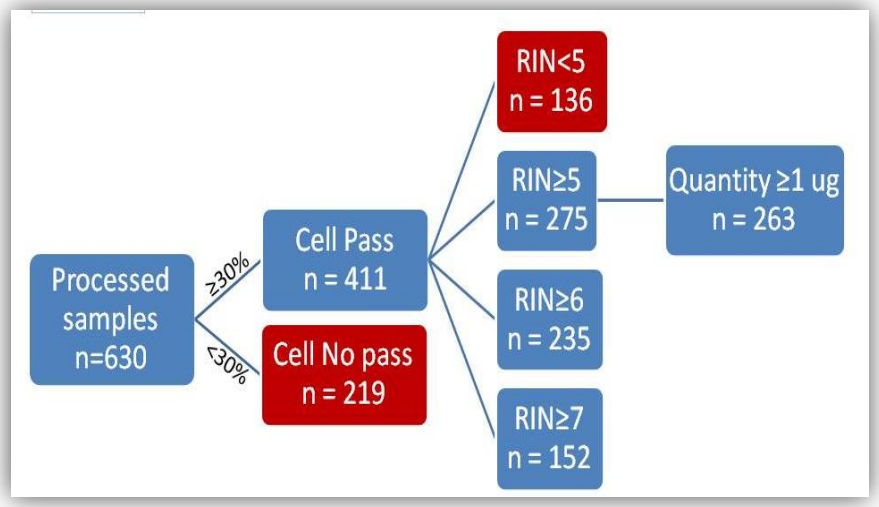
IN A MAJOR TRIAL

IN A MAJOR TRIAL



IN A MAJOR TRIAL





Region	Baseline samples processed	% RNA cell pass	% RIN pass
Southern Africa	37	65	71
Eastern Asia	119	78	72
Southern Asia	23	**30**	**14**
Europe	340	66	72
Latin America	110	**55**	**46**
Canada	1	100	100
Overall	**630**	**65**	**67**

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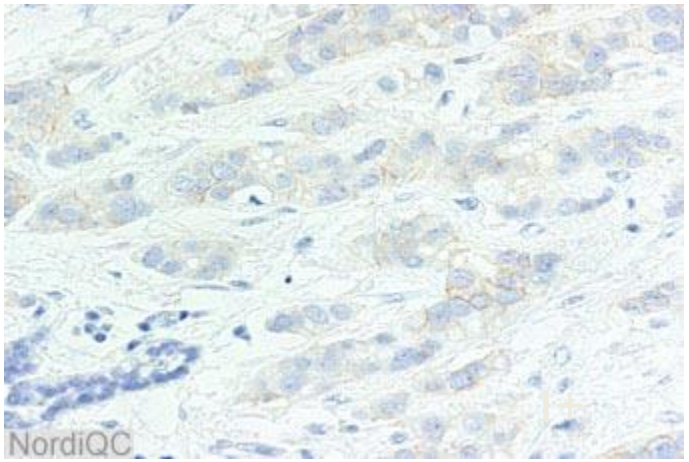
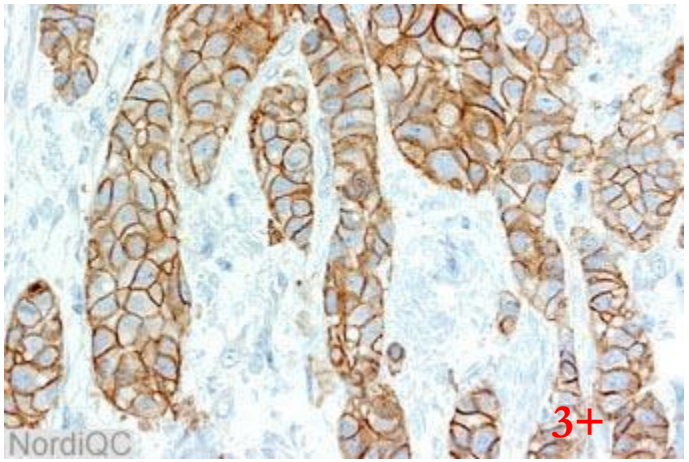
Unpublished observations

Is time from sampling till freezing or fixation important?

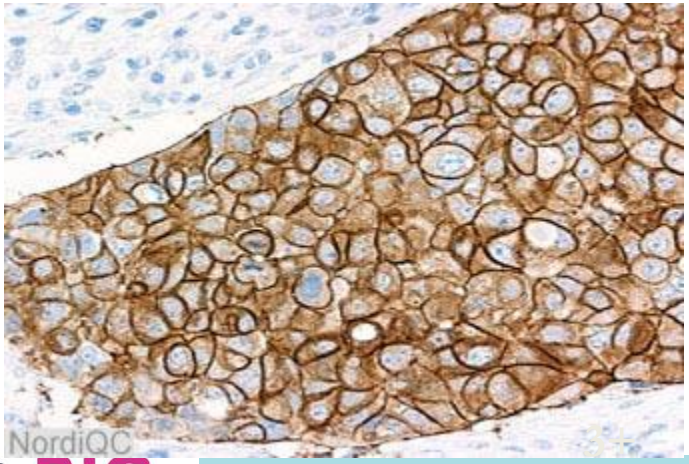
IHC errors

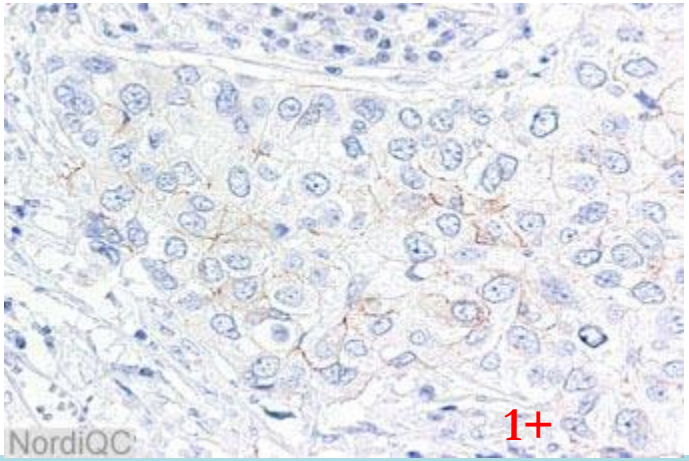
Tumour without gene amplification



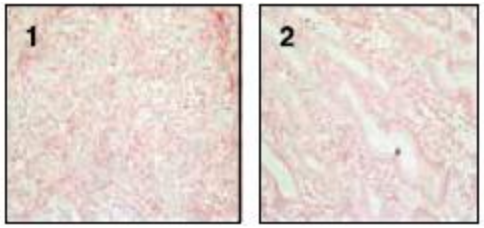


Tumour with gene amplification

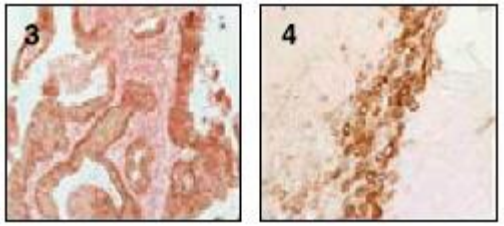




Stability of pAkt in tumor tissue: surgery vs. biopsy



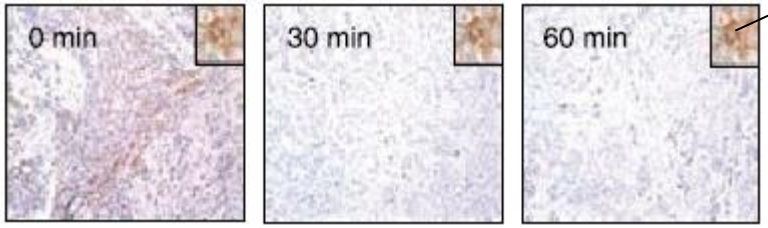
surgery



biopsy

HT–29 human tumor xenografts

Kept at RT for:

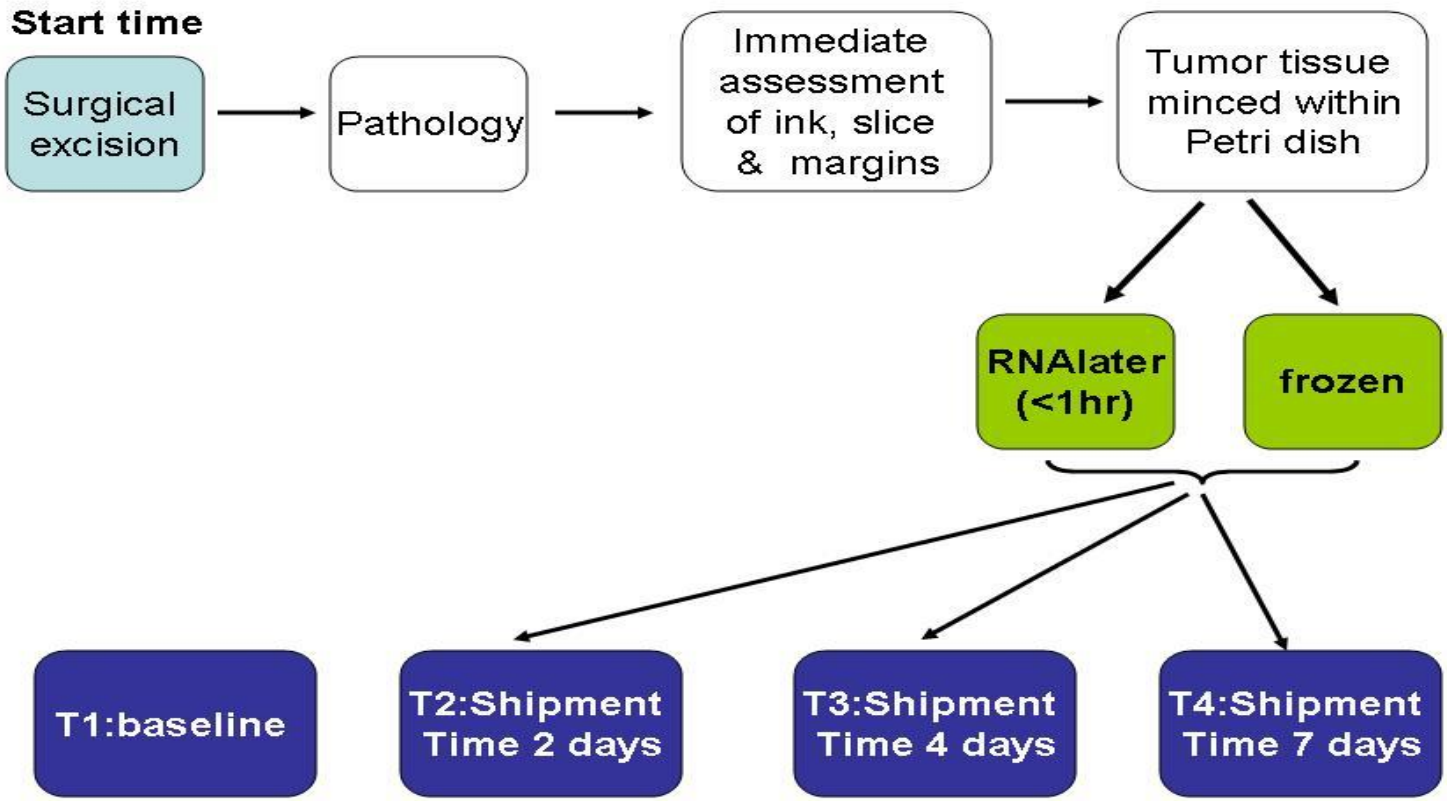


pAkt in HT-29 cells

From Baker et al. (2004) Clin Cancer Res 11(12): 4338-4340

Are storage conditions important?

Effect of sample preservation method and transportation duration on tumor gene expression profiling in breast cancer.



• **RNA degradation was significantly higher over time for samples preserved in RNAlater compared with frozen** (difference in changes 0.68,CI=0.49 to 0.87,p<0.001 and 0.33,0.25 to 0.4,p<0.001, respectively).

• **Genes and gene modules that were significantly differently expressed over time, independent of preservation method, were AURKA, PTEN, CASP3 and WOUND** (range of 1-day changes in log2 expression:-0.05 to 0.1, p<0.01), while the expression of ESR1 and ERBB2 was unaffected.

• **Genes that were significantly influenced by sample preservation method, adjusted for time, were ESR1, PLAU, VEGF, PIK3CA, PTEN and gene modules GENE21 and GENE70 was** (range: -1.03 to 0.16, p<0.01).

• Using **PAM50**, 8 (**61%**) samples were classified at least once as a **different** subtype over time compared to baseline.

• 4 (**30%**) samples fell in a **different GGI class risk** (high/low) at least once over time.

Take Home message 2

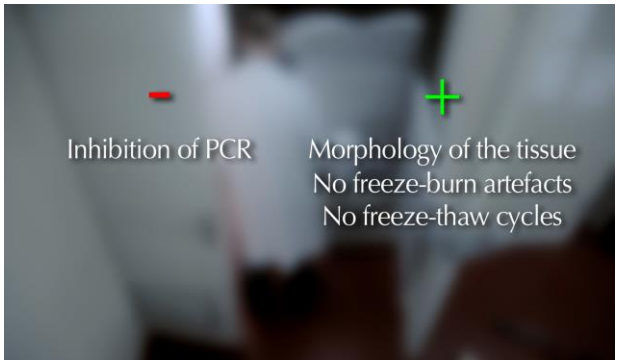
Time from sampling till freezing/ fixation is important

Method of storage and time of storage is important

That's why….

Improving translational research samples collection in international trials: the BIG-Movie

[This movie can be downloaded on www.bigagainstbreastcancer.org]



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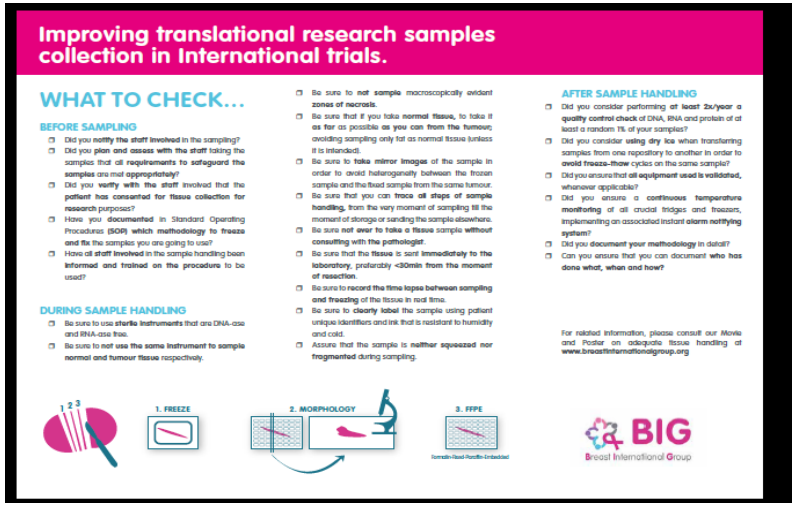
Thanks to University of Michigan. Breast Cancer research Foundation Grant

In addition, a poster and flyer have been developed, highlighting the main educational elements of the movie.



Thanks to University of Michigan. Breast Cancer research Foundation Grant

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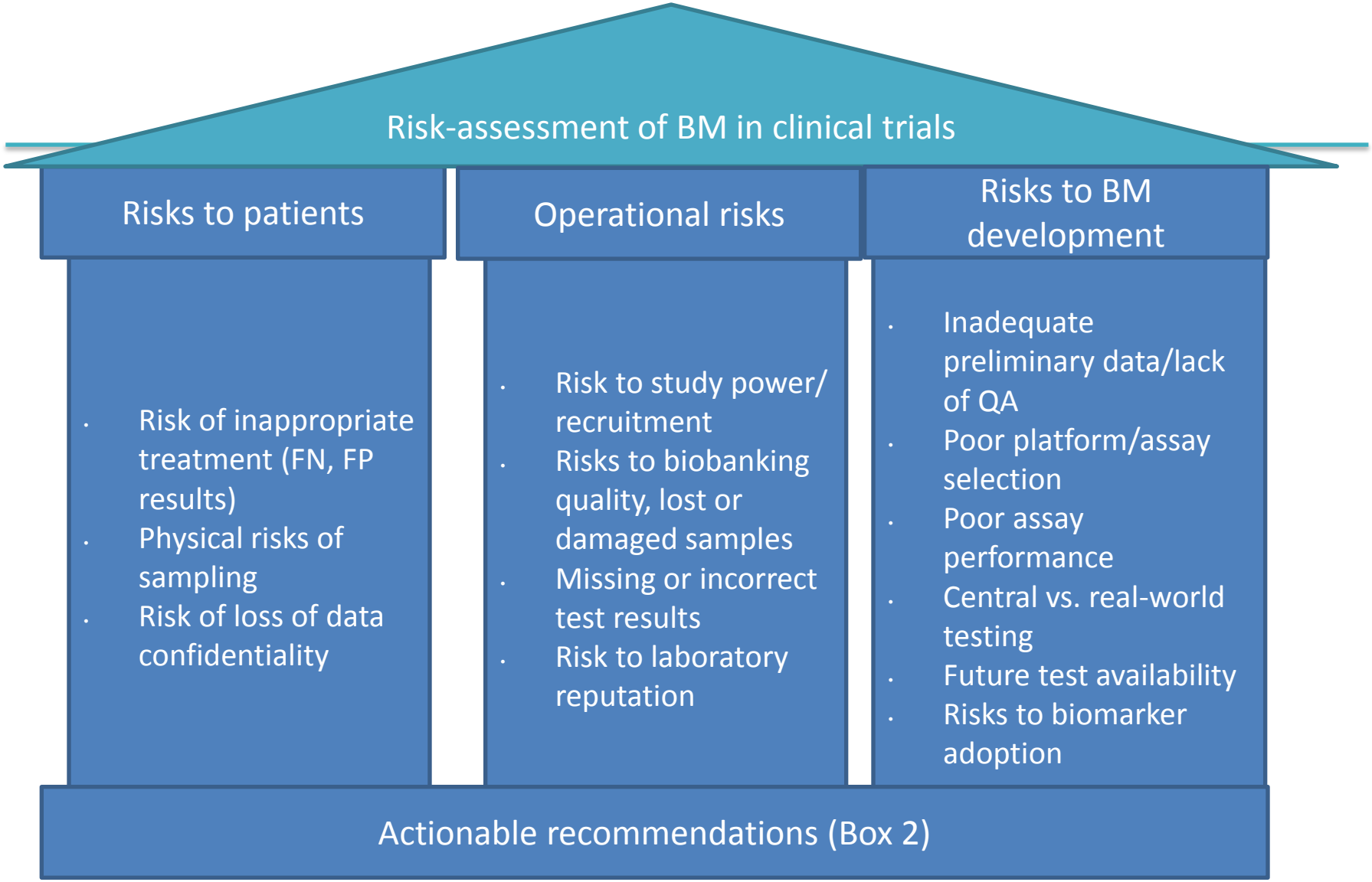
Why a risk-assessment strategy?

Which risks?

A risk-management approach for effective integration of biomarkers in clinical trials: perspectives of an NCI, NCRI, and EORTC working group

Jacqueline Anne Hall, Roberto Salgado, Tracy Lively, Fred Sweep, Anna Schuh



The three core pillars of risk-assessment of designing and executing clinical trials including biomarker assessment

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Hall J., Salgado R et al., Lancet Oncology 2014

OPERATIONAL RISKS

Risk	Example	Possible consequences	Solutions	Example
Failure to collect sufficient samples of adequate quality (collection, processing, shipping)	Missing samples. Unidentifiable samples or samples missing necessary annotation. Samples that fail to return conclusive assay results	Missing, unidentifiable or poor quality samples can lead to missing or unreliable test results, for real-time, integral marker assessment this can affect patient randomization. Legal uncertainties can prevent sharing of samples. Poor inter-lab concordance can lead to poor study power or bias in the type of samples analyzed. Frequent shipments increase costs, too infrequent jeopardize study operations. Batching analysis of samples can lead to reproducibility concerns/batch effects on test results.	Include sample and test requirements in site selection (e.g. in site feasibility assessment), develop expert networks with demonstrated expertise/ facilities, work with QMS certified sites, master agreements with sites managing local governance rules, QA of samples/tissue performed by a pathologist. Dummy-runs for real-time testing (e.g. using cell line material), SOPs and workflow management can reduce errors. Courier services should be regularly reviewed for performance. Sample requirements clearly stated in the protocol. Appropriate communication with pathologists and establishment of clear accountability and traceability of the tissues within the trial.	TransBIG Biobank in MINDACT trial. Biobanking, histology and quality review EORTC Brain Group trials.

Recommandations in protocol design

Protocols must include:

- Evidence of early engagement with experts from relevant disciplines for protocol development including pathologists, biobank experts, translational scientists, medical oncologists, and biostatisticians.

- Evidence of the biobank(s) experience/certification, the banking process, the designated providers of samples, evaluation of sample integrity, availability and quality.

Recommandations for during the study

- Inter and intra-run / inter-lab variability and other trends should be closely monitored and any required changes must be in protocol amendments.

- This also includes:
  - QA of on-going sample banking,
  - QA of on-going assay performance,
  - Continuous monitoring of the number of available samples,
  - Interim analysis to re-assess feasibility of approach and whether statistical power will still be reached,
  - Appropriate data management and QA of BM data,
  - Interim evaluation of the likelihood that BM data will be submitted to support regulatory approval.

Recommandations for after the study

- Explore potential routes to biomarker adoption, including commercialization, test provision, design of further clinical trials, necessary improvements in the method, etc.

- Implement processes for accessing banked samples for future research or bridging studies to the final commercial assay.

Take Home message 3

A risk-assessment approach

can be very useful to protect in a preventive manner

our patients from any “errors”

Thank you