

SAMPLE COLLECTION AND PROCESSING

A Risk Management Approach

IMPAKT 2014
Roberto Salgado

Disclosures

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

The sample flow in a Pathology Laboratory

MACROSCOPY



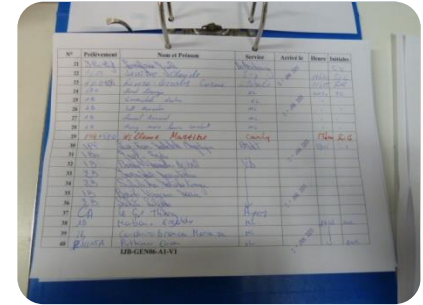
RECEPTION



LABELING



REGISTRATION



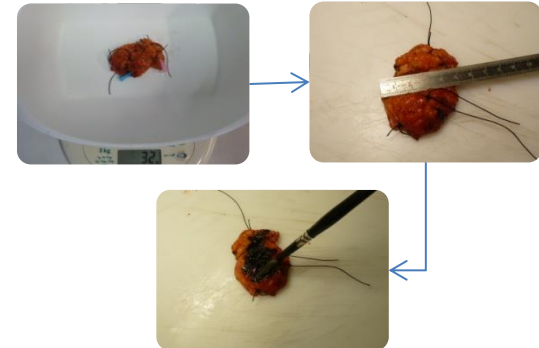
VIP



TISSUE HOLDER



TISSUE DESCRIPTION

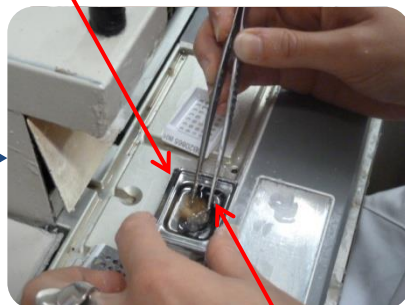


INCLUSION IN PARRAFIN

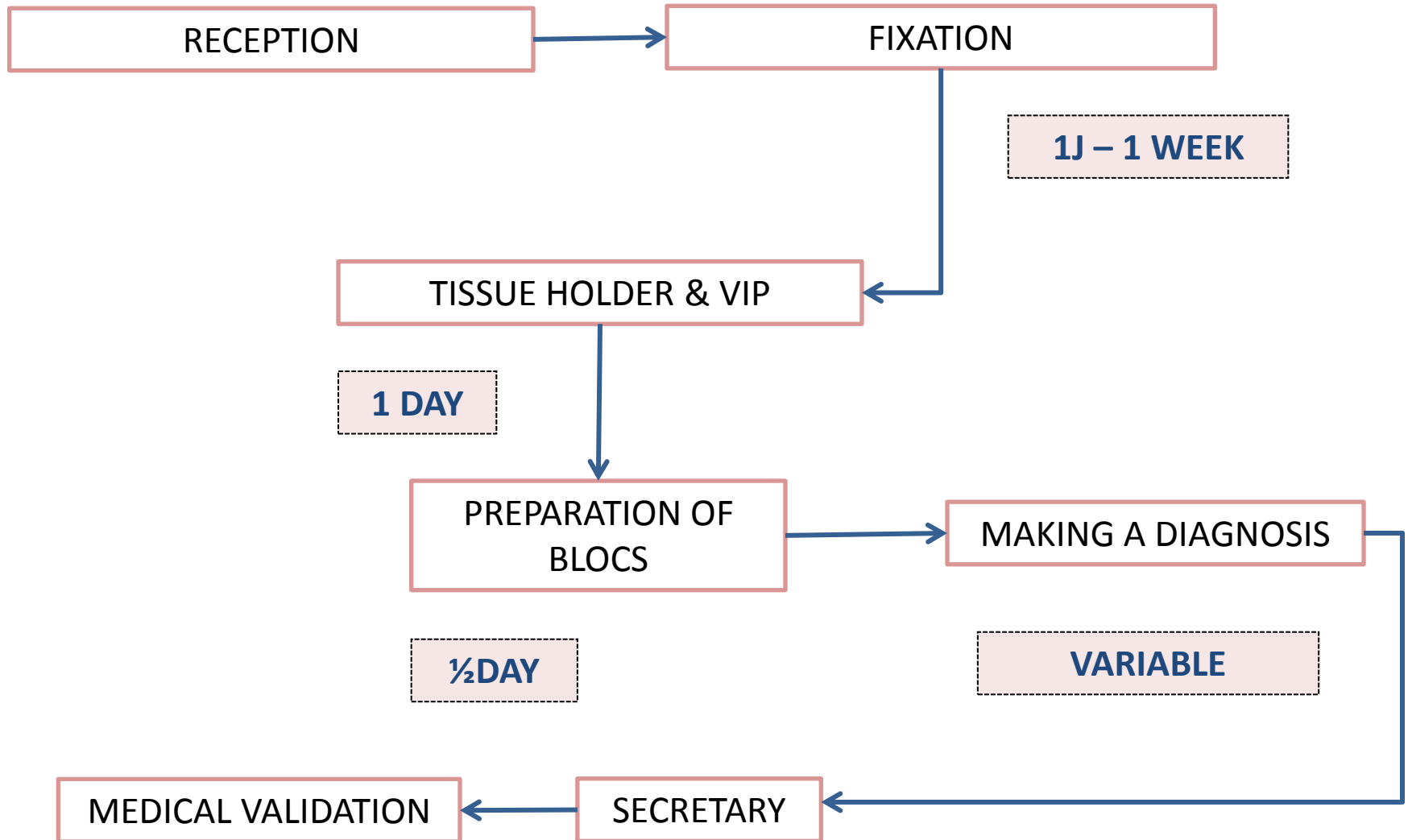
**METALLIC
HOLDER**

COLD TABLE

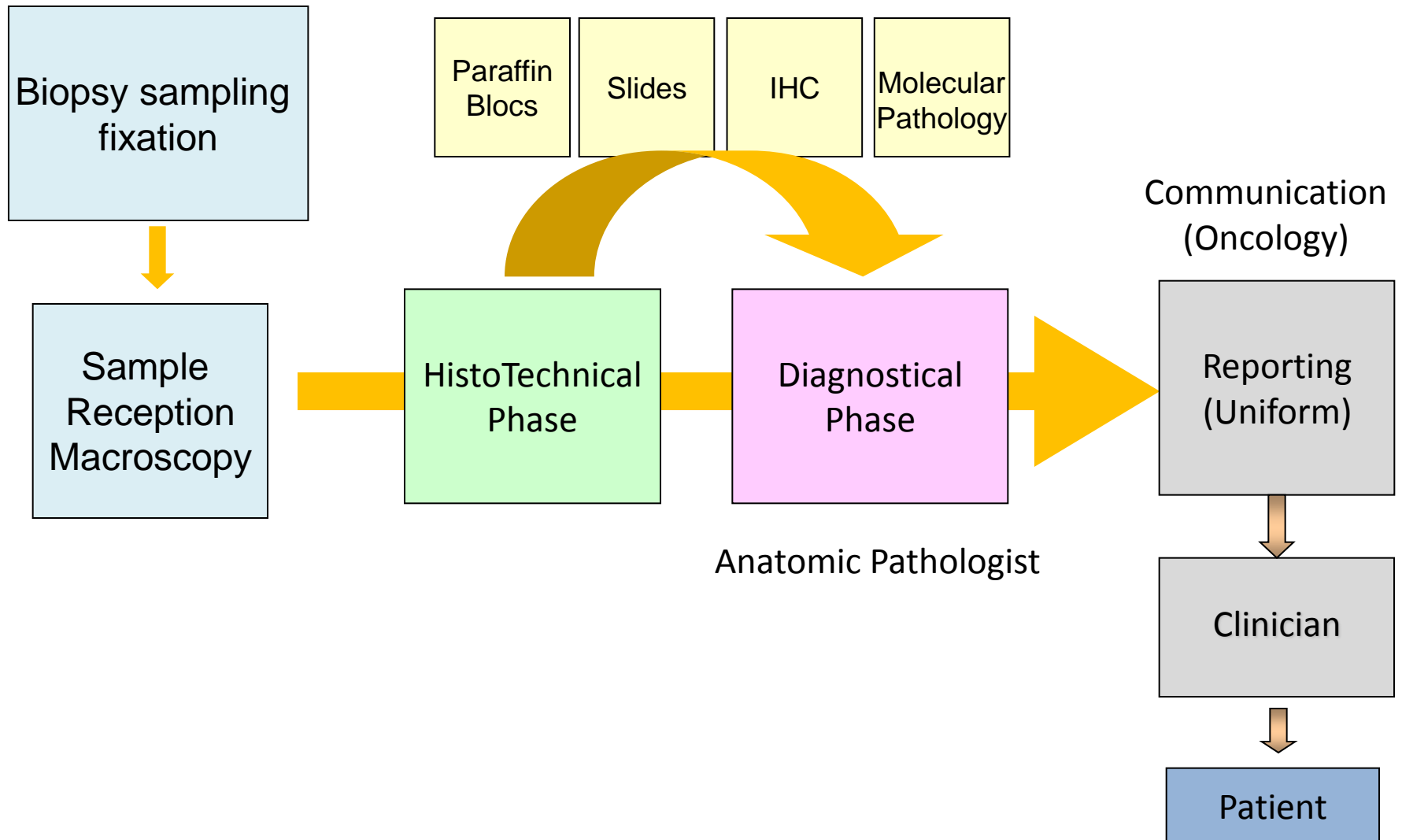
LIQUID PARRAFIN

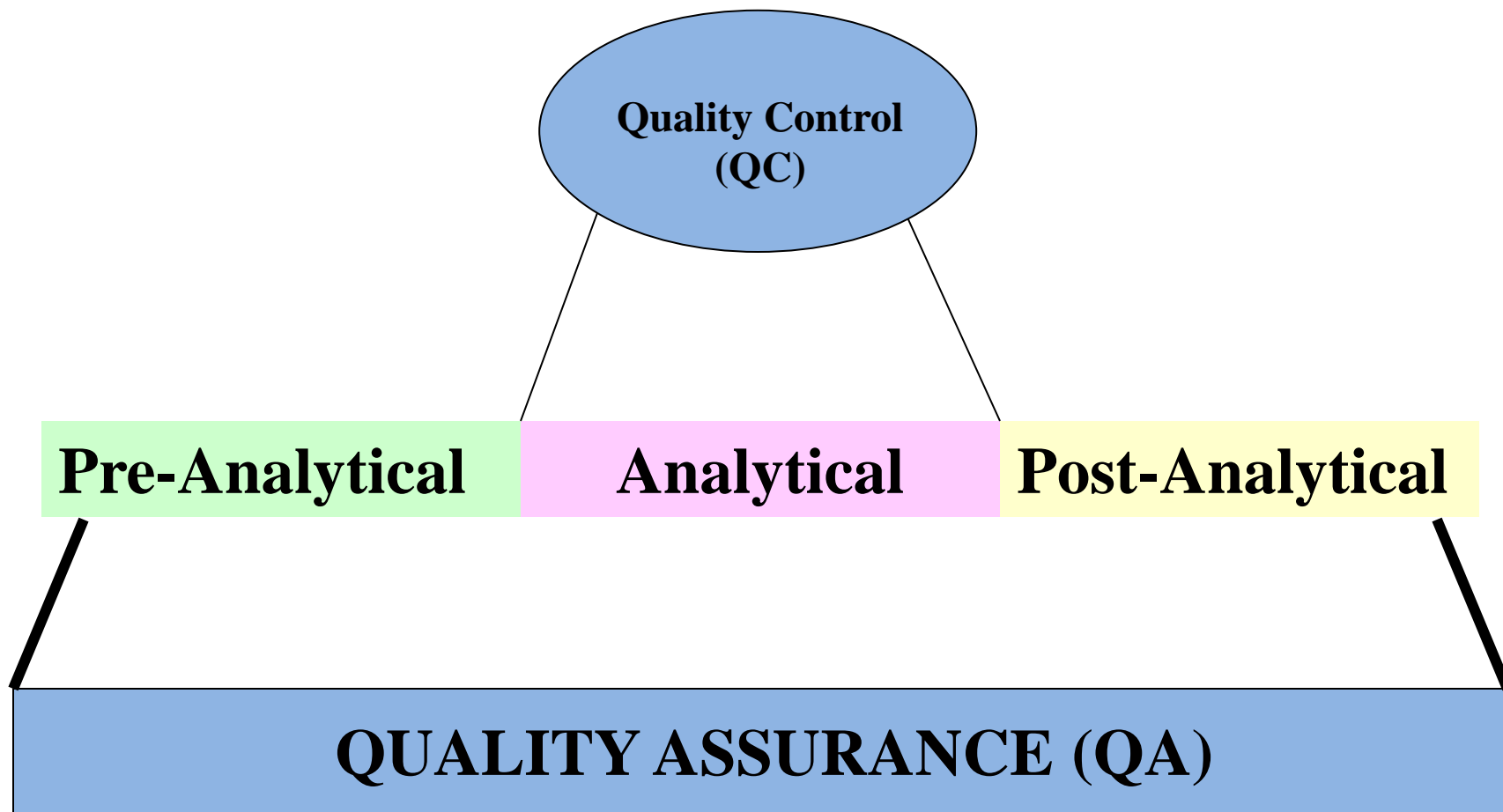


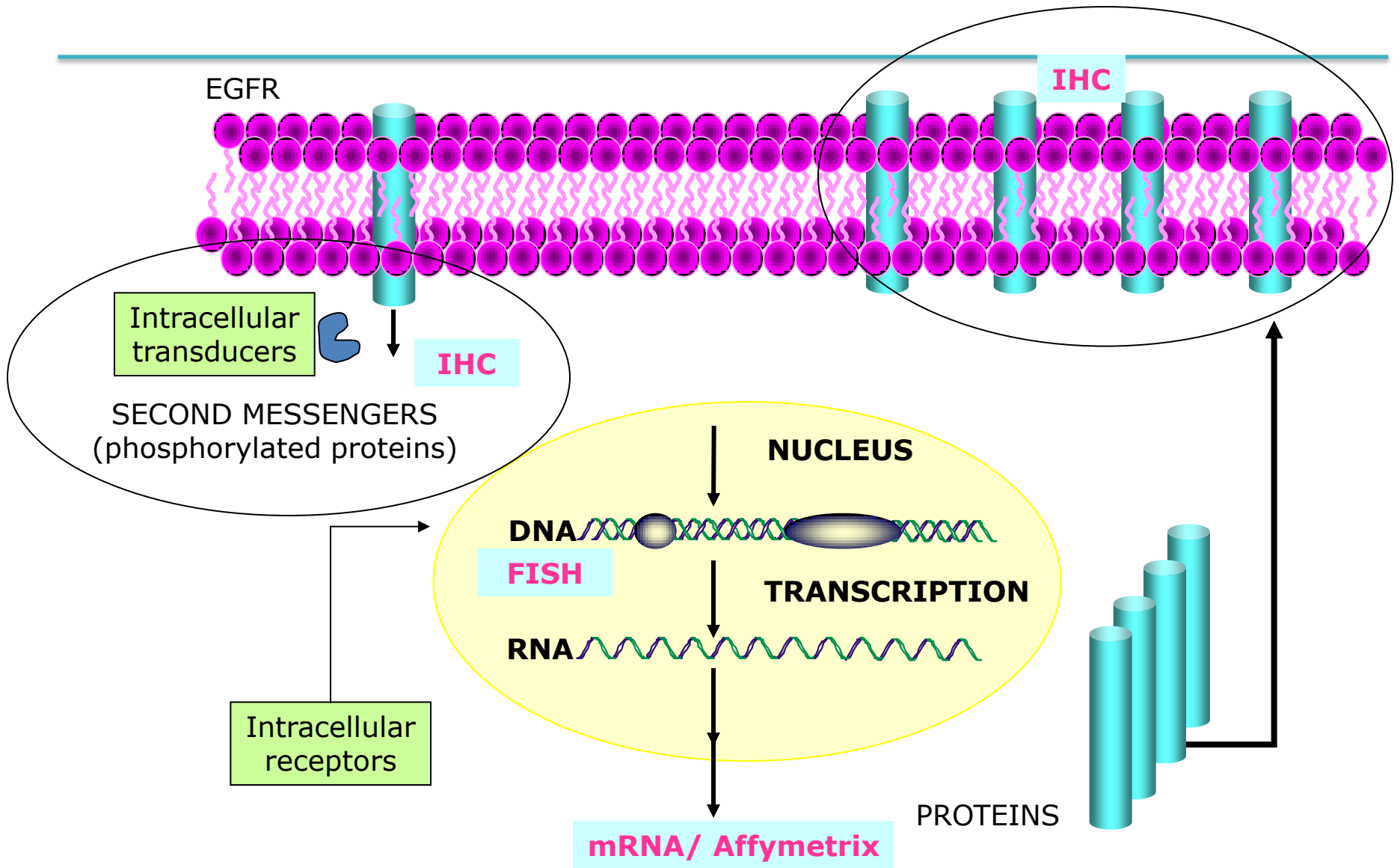
FROM SAMPLE RECEPTION TO DIAGNOSIS



THE PATHOLOGY WORK FLOW







Take Home message 1

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

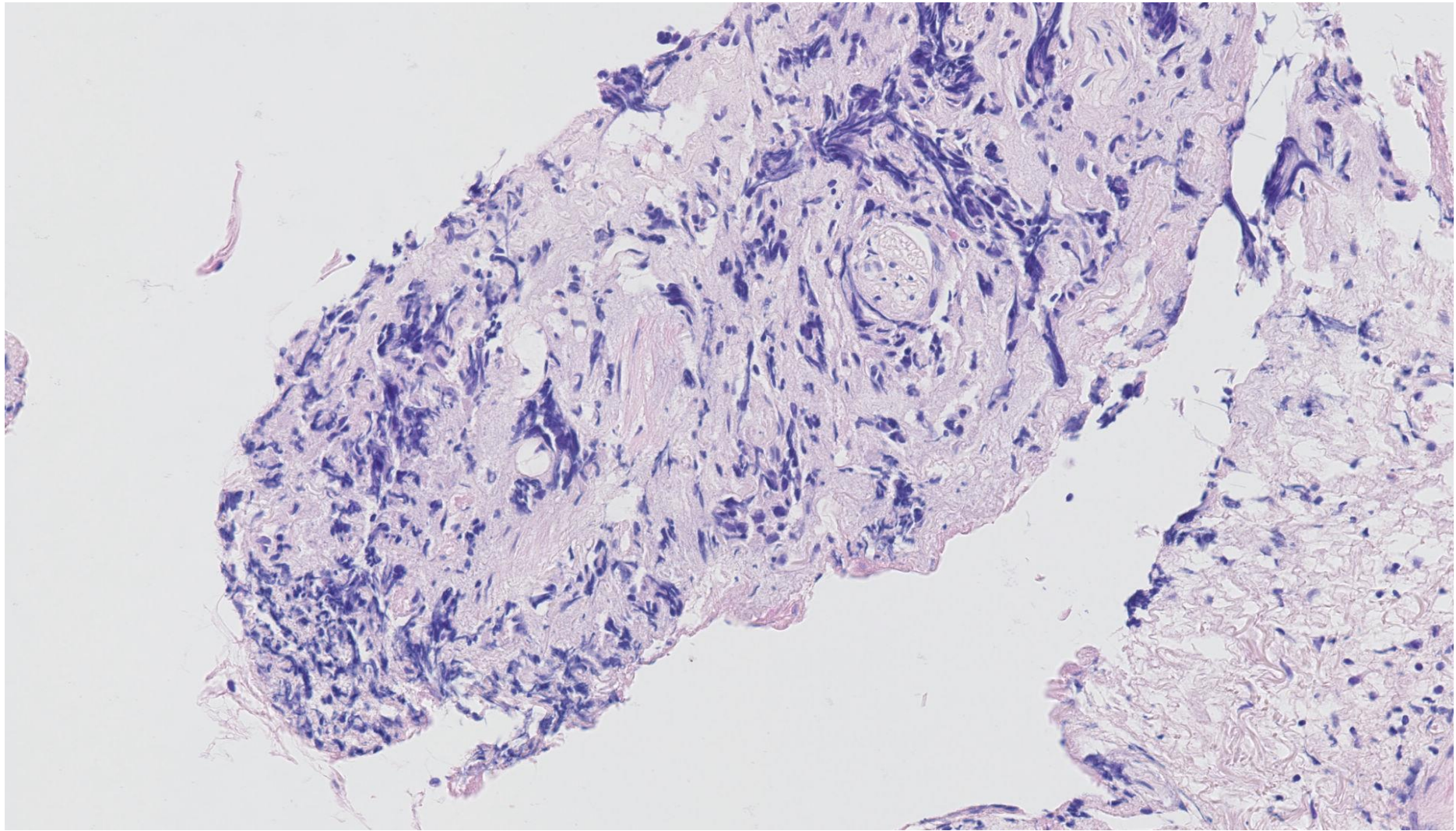
Why is good sample handling important?

A few examples

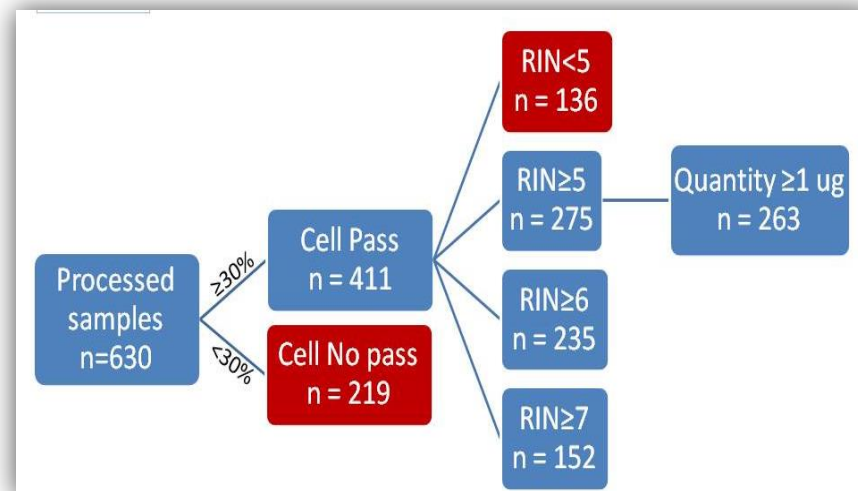
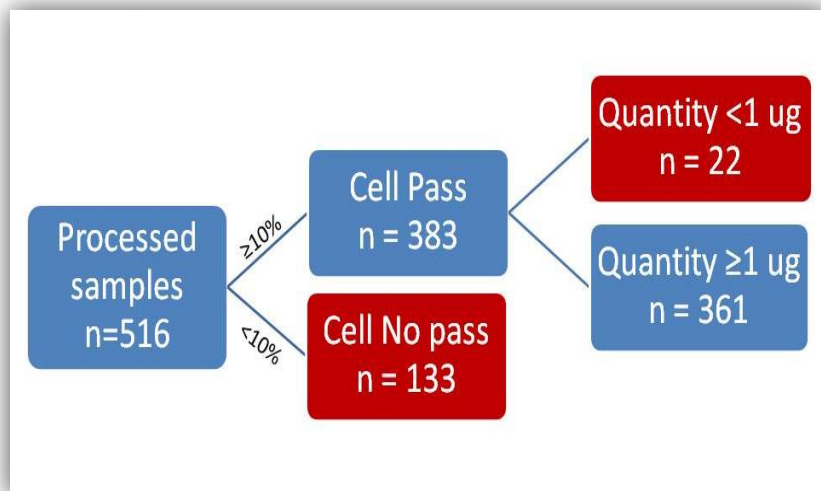
IN A MAJOR TRIAL



IN A MAJOR TRIAL



IN A MAJOR TRIAL

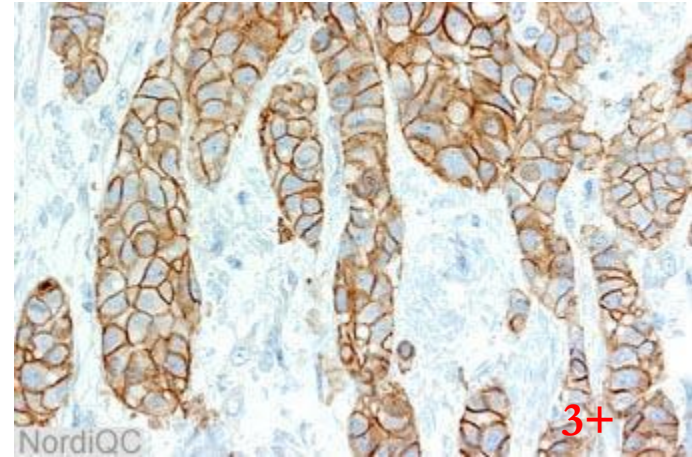
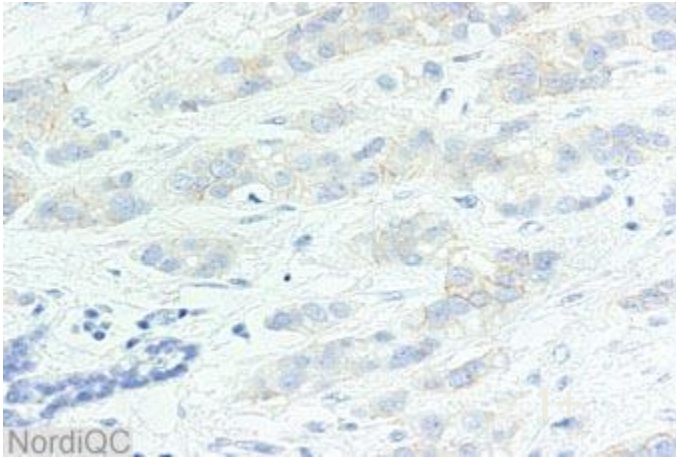


Region	Baseline samples processed	% RNA cell pass	% RIN pass
Southern Africa	37	65	71
Eastern Asia	119	78	72
Southern Asia	23	30	14
Europe	340	66	72
Latin America	110	55	46
Canada	1	100	100
Overall	630	65	67

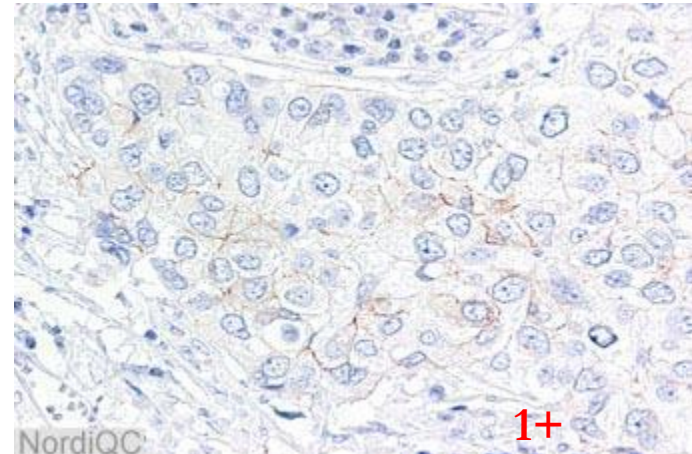
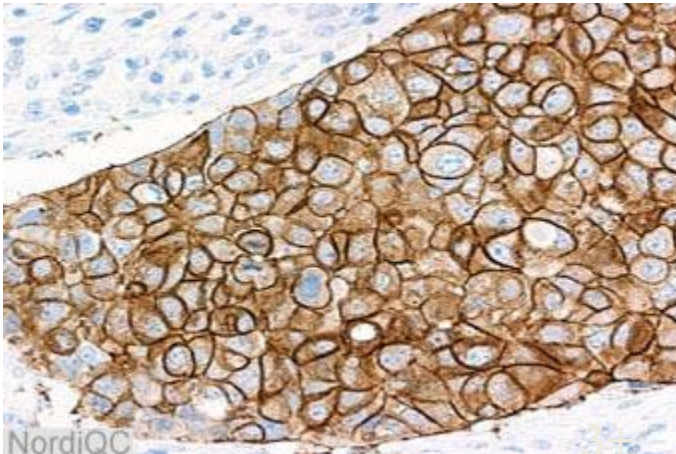
Is time from sampling till freezing
or fixation important?

IHC errors

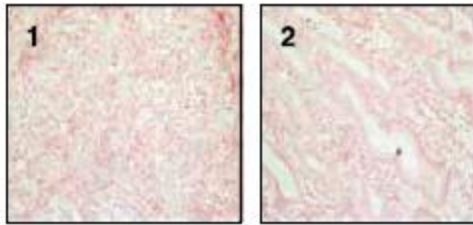
Tumour without gene amplification



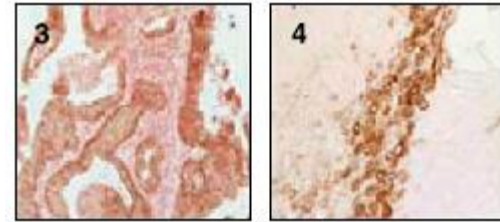
Tumour with gene amplification



Stability of pAkt in tumor tissue: surgery vs. biopsy



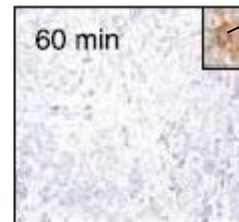
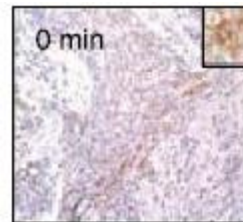
surgery



biopsy

HT-29 human tumor xenografts

Kept at RT for:

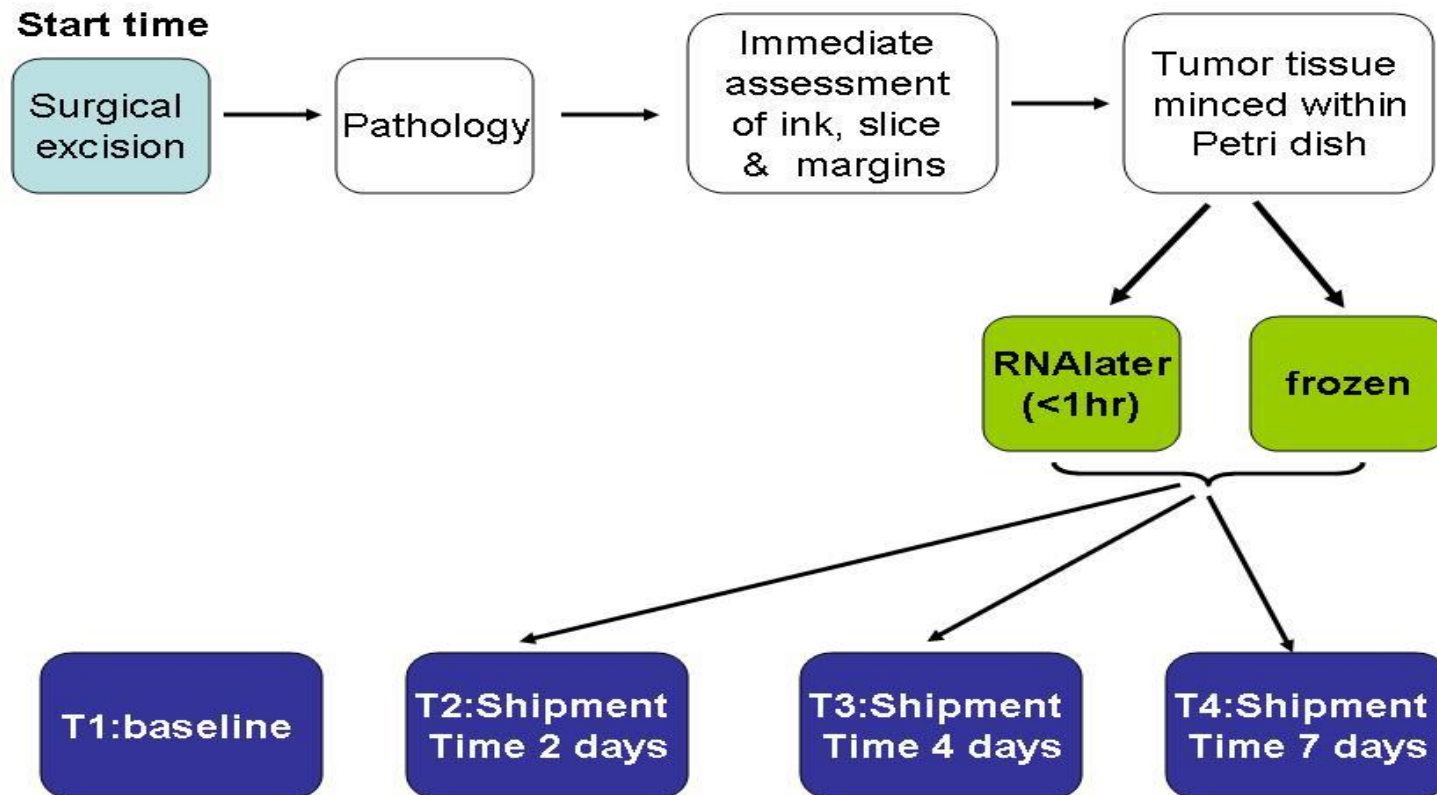


pAkt in HT-29 cells

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

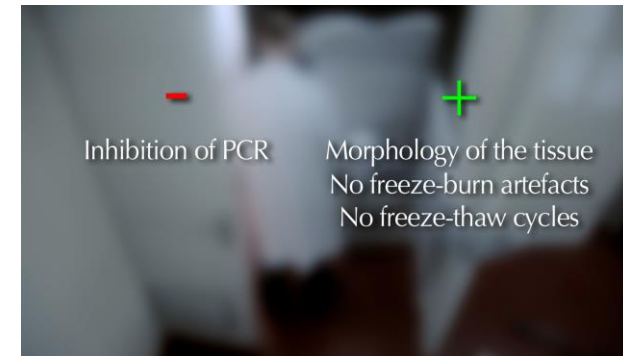
Method of storage and time of storage is important

That's why....

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



This movie can be downloaded on
www.bigagainstbreastcancer.org



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

This checklist lists the main items that need to be checked when handling samples for international trials.

Improving translational research samples collection in international trials

DISCLAIMER

The white sheet is provided "as is" (without warranty) in terms of handling samples in clinical cancer trials. It is not, and has never put in the past, legally used methods, knowing that other methodologies and procedures may vary also in some or various laboratories. Its completion is made with other methodologies and procedures. Software. This Breast International Group (BIG) will not be liable in any way with regard to the application of any content of the present sheet.

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 And all members of the IISG Executive Board



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 for more info consult our poster or movie
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Improving translational research samples collection in International trials.

WHAT TO CHECK...

BEFORE SAMPLING

- Did you verify the staff involved in the sampling?
- Did you plan and assess with the staff training the samples that all requirements to safeguard the samples are met appropriately?
- Did you verify with the staff involved that the patient has consented for tissue collection for research purposes?
- Have you documented in Standard Operating Procedures (SOP) which methodology to freeze the samples you are going to use?
- Have all staff involved in the sample handling been informed and trained on the procedure to be used?

DURING SAMPLE HANDLING

- Do you use to use extra instruments that are DNA-safe and RNA-safe too.
- Do you use to use the same instrument to sample normal and tumour tissue respectively.
- Do you not sample macroscopically evident zones of necrosis.
- Do you make that if you take normal tissue, to take it as far as possible as you can from the tumour, avoiding sampling only fat or normal tissue besides it is intercalated.
- Do you make to take minor images of the sample in order to avoid heterogeneity between the frozen sample and the final sample from the same tumour.
- Do you make that you can freeze all steps of sample handling, from the very moment of sampling till the moment of storage or sending the sample elsewhere.
- Do you not stop to take a tissue sample without consulting with the pathologist?
- Do you make that the tissue is sent immediately to the laboratory, preferably <30min from the moment of resection.
- Do you make to record the time lapses between sampling and freezing of the tissue in real time.
- Do you make to clearly label the sample using patient unique identifiers and link that is resistant to humidity and cold.
- Assure that the sample is neither squeezed nor fragmented during sampling.

AFTER SAMPLE HANDLING

- Did you consider performing at least 2x/yr a quality control check of DNA, RNA and protein of at least a random 1% of your samples?
- Did you consider using dry ice when transferring samples from one repository to another in order to avoid freeze-thaw cycles on the same sample?
- Did you ensure that all equipment used is validated, whenever applicable?
- Did you ensure a continuous temperature monitoring of all evoked ridges and reservoirs, implementing an associated internal alert notifying system?
- Did you document your methodology in detail?
- Can you ensure that you can document what has done what, when and how?

For related information, please consult our [Manual](#) and [Poster](#) on adequate tissue handling of www.breastinternationalgroup.org

1. FREEZE

2. MORPHOLOGY

3. FREEZE

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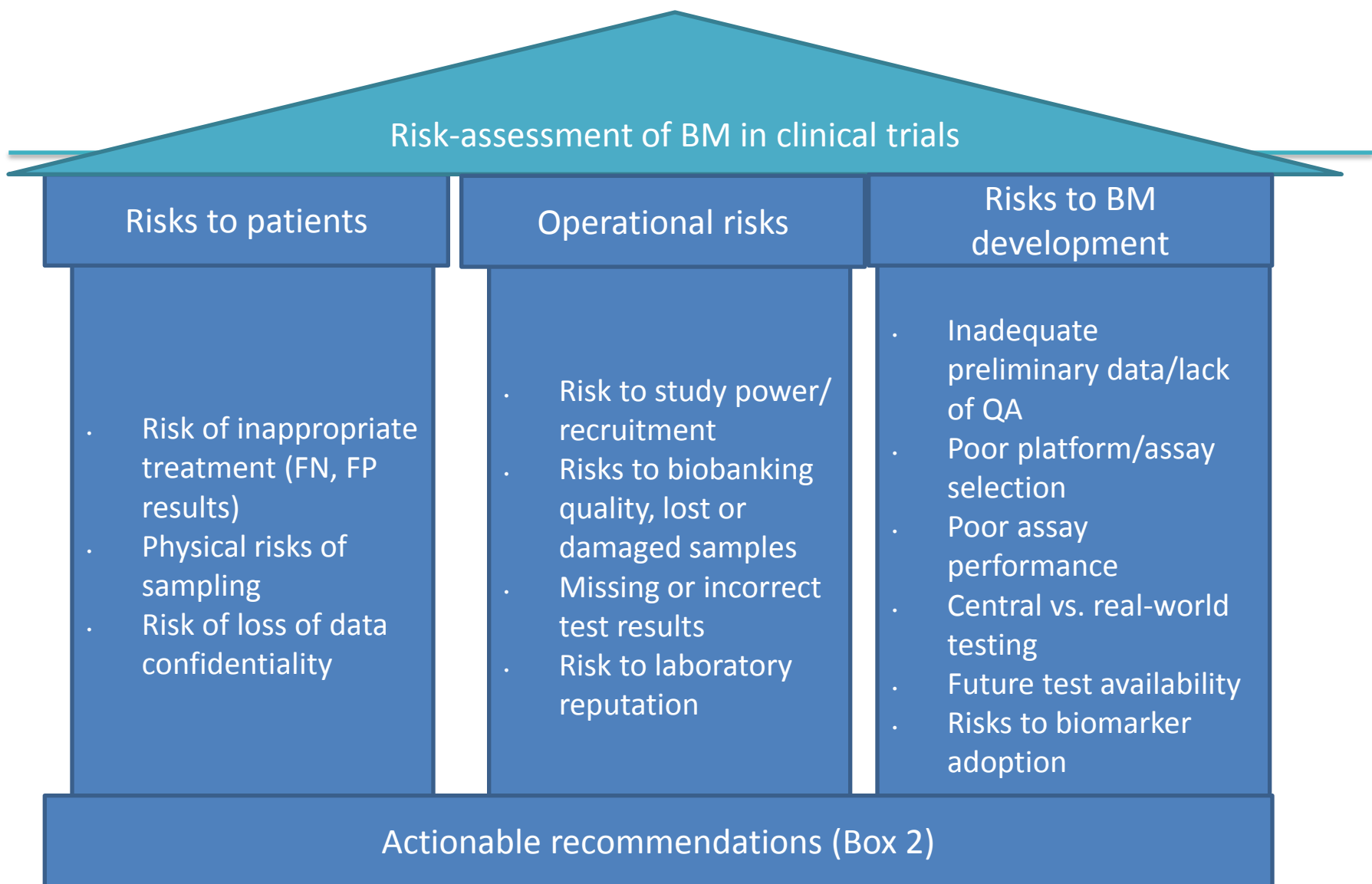
Why a risk-assessment strategy?

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

OPERATIONAL RISKS

Risk	Example	Possible consequences	Solutions	Example
Failure to collect sufficient samples of adequate quality (collection, processing, shipping)	<p>Missing samples.</p> <p>Unidentifiable samples or samples missing necessary annotation.</p> <p>Samples that fail to return conclusive assay results</p>	<p>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.</p> <p>Legal uncertainties can prevent sharing of samples.</p> <p>Poor inter-lab concordance can lead to poor study power or bias in the type of samples analyzed.</p> <p>Frequent shipments increase costs, too infrequent jeopardize study operations.</p> <p>Batching analysis of samples can lead to reproducibility concerns/batch effects on test results.</p>	<p>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.</p> <p>Dummy-runs for real-time testing (e.g. using cell line material), SOPs and workflow management can reduce errors.</p> <p>Courier services should be regularly reviewed for performance.</p> <p>Sample requirements clearly stated in the protocol.</p> <p>Appropriate communication with pathologists and establishment of clear accountability and traceability of the tissues within the trial.</p>	<p>TransBIG Biobank in MINDACT trial.</p> <p>Biobanking, histology and quality review EORTC Brain Group trials.</p>

Recommendations 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.

Recommendations 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.

Recommendations 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