7th Diagnostic Days – Düsseldorf, 2013

Accreditation for Molecular Pathology Laboratories
Today and Tomorrow

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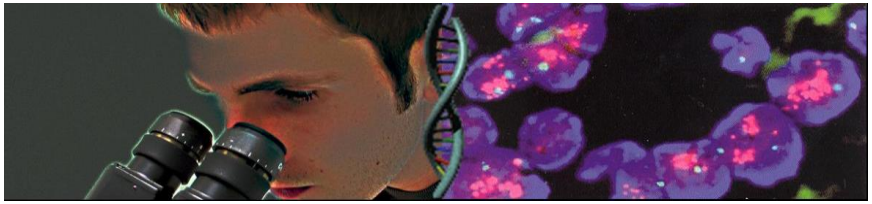
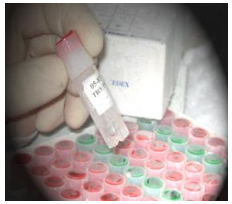
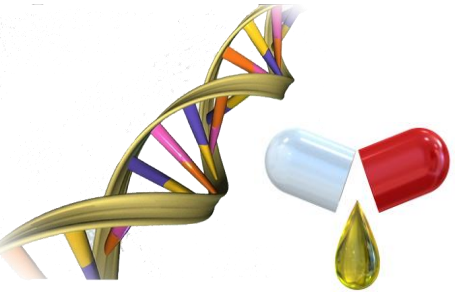
University of Nice Sophia Antipolis, France



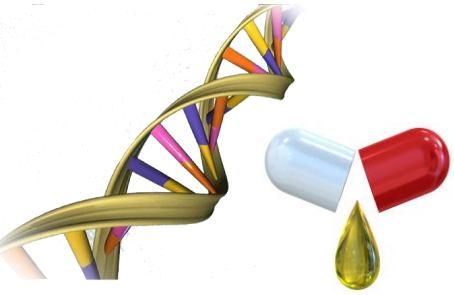


QIAGEN would like to thank our speaker, Prof. Paul Hoffman, for his presentation.



Agenda

- Current challenge
- The times have changed: molecular pathology is here to stay
- The ISO 15189:2007 standard
- Main bottlenecks and how to be successful ?
- The French organisation for molecular pathology testing in personalised medecine
- External Quality Control programme in France
- End-points



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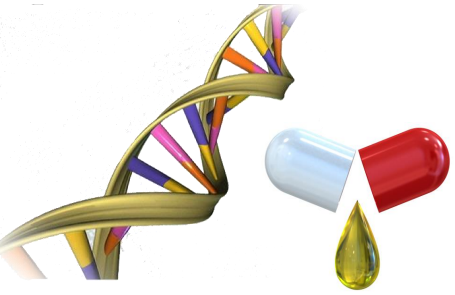
Targeted therapy in clinical oncology practice

New major opportunities for cancer patients

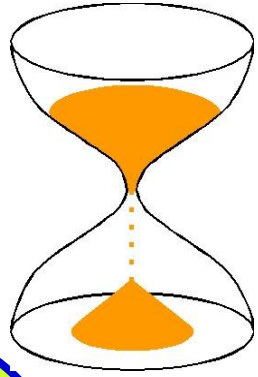
Development of cancer biomarkers and companion diagnostics for personalised oncology



- Molecular diagnostic industry is in a state of rapid evolution

- Continuous technology developments and new clinical opportunities for drug selection

- Molecular diagnostic field is a major component of diagnostic industry to date !



The right drug

The right patient

The right time

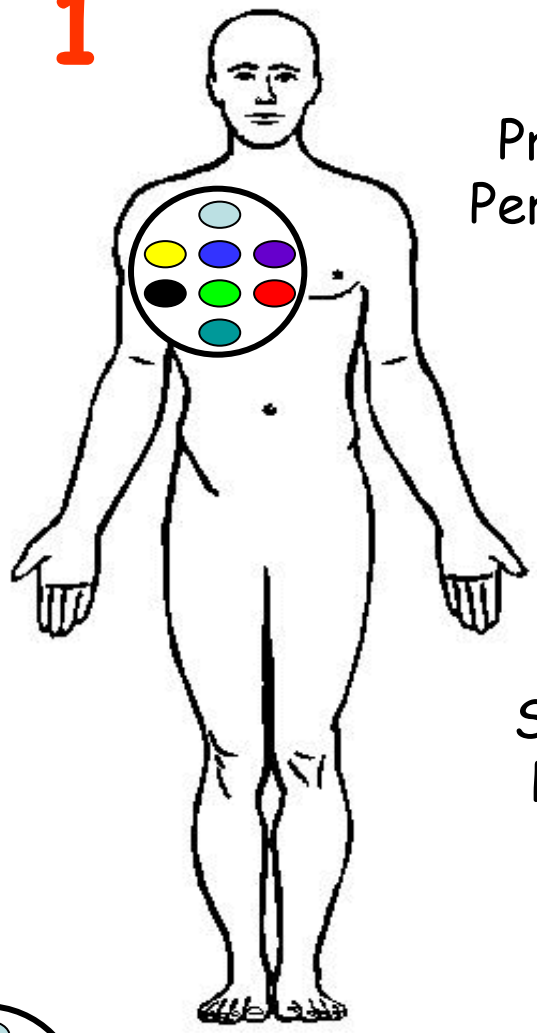
Personalised Health Care
Cancer Patient

The right approach

The right cost

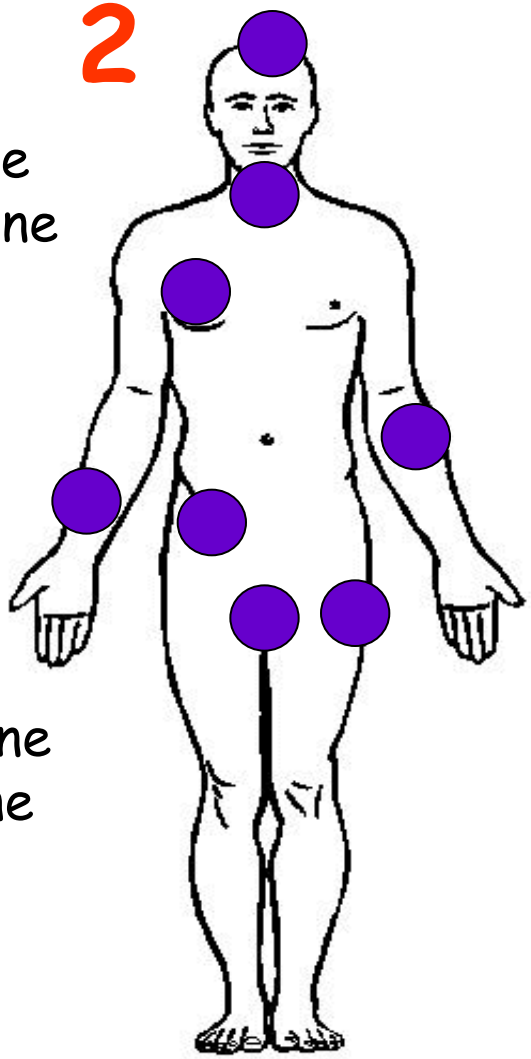
Two concepts

1

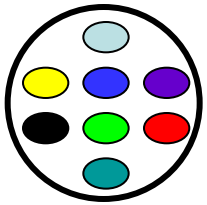


Predictive medecine
Personalised medecine

2



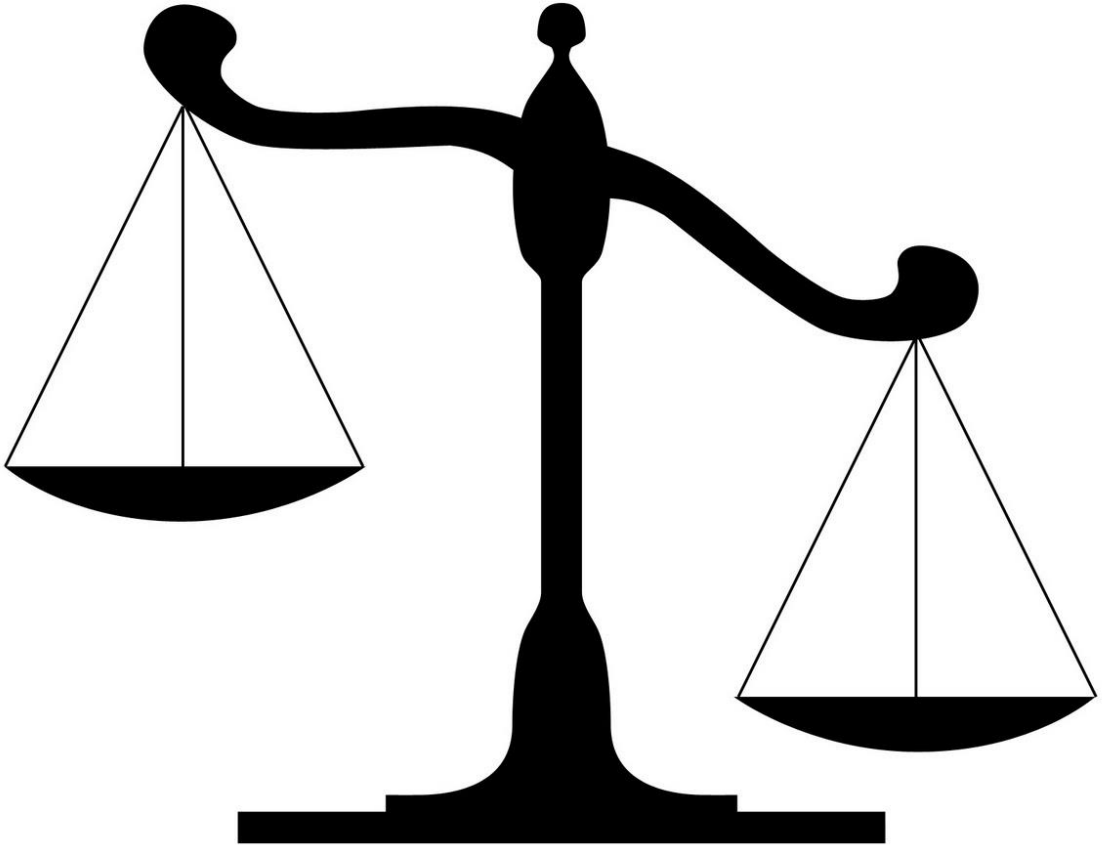
Stratified medecine
Precision medecine



One cancer (ex lung carcinoma),
Many genomic alterations

Many cancers, one genomic alteration
(ex *BRAF V600E*)

The molecular diagnostic tests need a high degree of Quality Assurance and Quality Control



Assessment of the benefit-risk balance for each targeted therapy

We have to keep in mind

A false negative result:

Loss of chance to get an effective treatment as a result of false negative molecular tests

A false positive result:

Needlessly be exposed to the risk associated with the treatment as a result of false-positive molecular tests

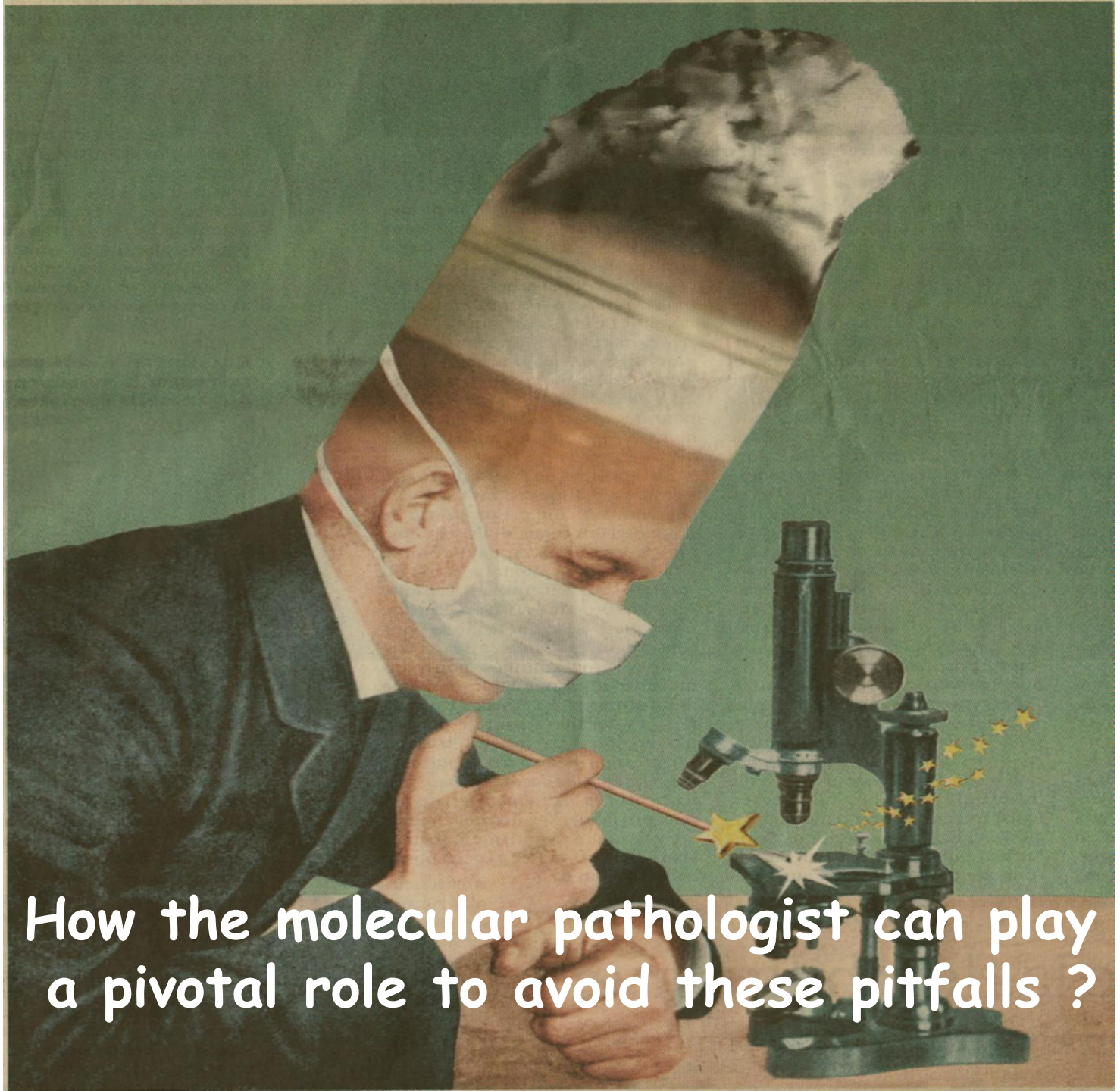
How the reduce the risk ?

Patients lodging complaints of medical malpractice ?

Fact of fancy (in Europe) for a wrong molecular test result in 2013 ?

How pitfalls in molecular biology be better anticipated ?

Ilie and Hofman, Current Med Chem, 2012

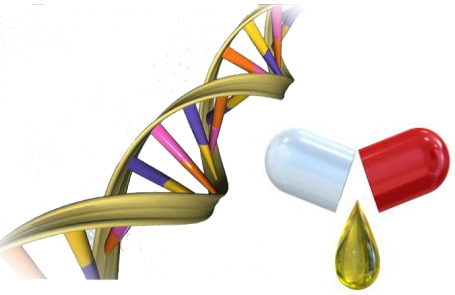

How the molecular pathologist can play
a pivotal role to avoid these pitfalls ?

Quality **A**ssurance & **Q**uality **C**ontrol

=

Laboratory **A**ccreditation

ISO 15189 **R**eferential [Europe]



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« Molecular Biology » and « Molecular Pathology »

Is it the same job ?

Biologist

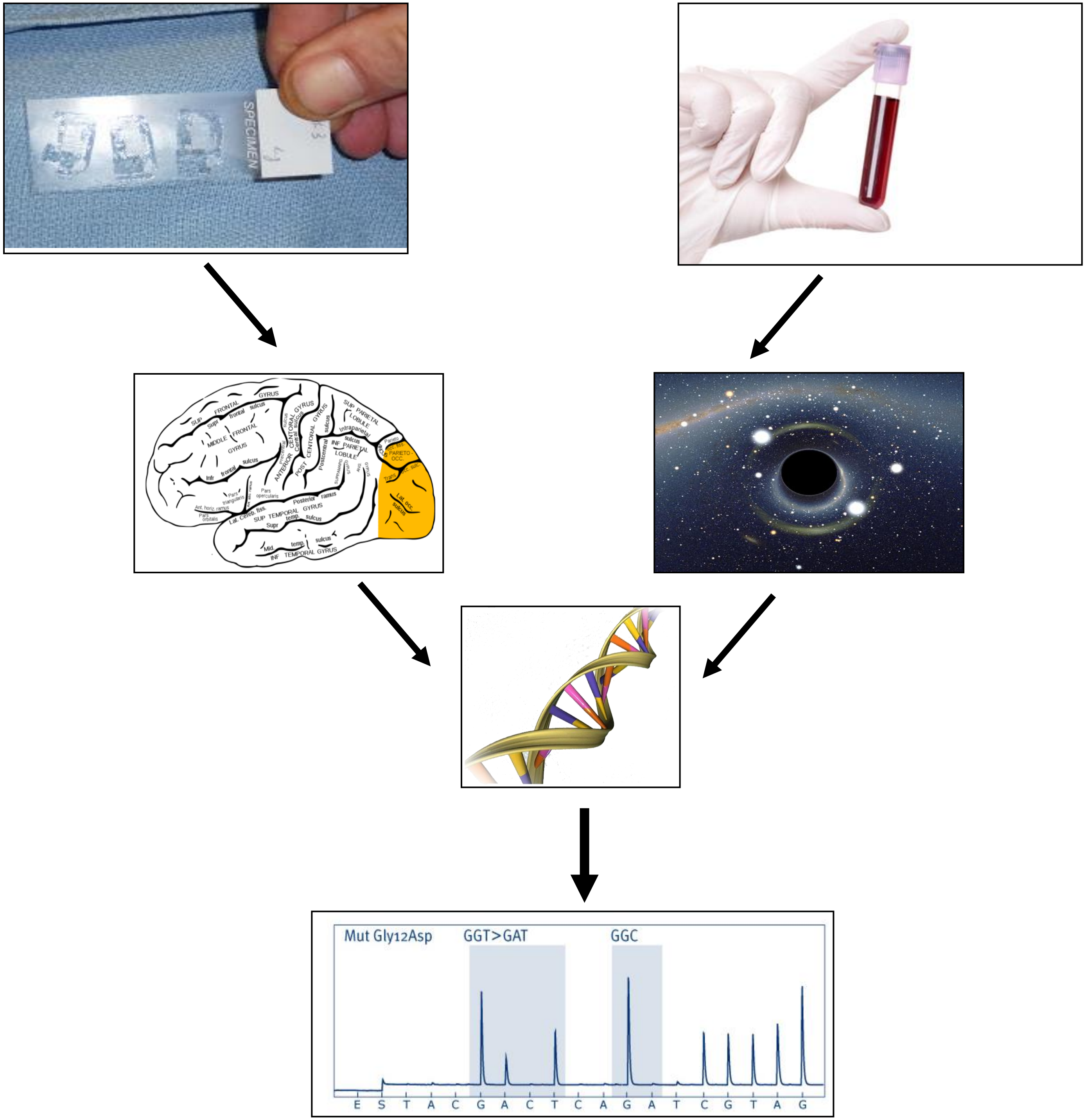
Clinical Pathologist

A controversial issue !

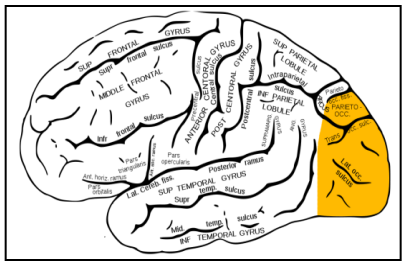
Probably not !

« Molecular Biology » and « Molecular Pathology »

Is it the same job ?



SPECIMEN
Mut Gly12Asp
GGT>GAT
GGC
E S T A C G A C T C A G A T C G T A G



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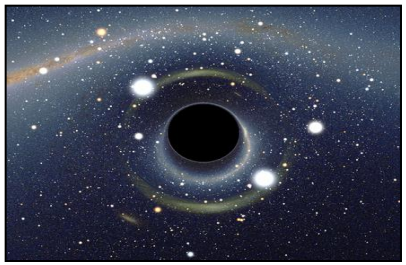
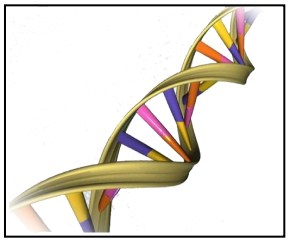


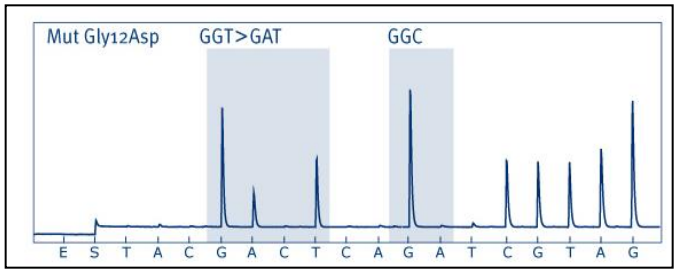
« Visual expertise »

« Black hole »



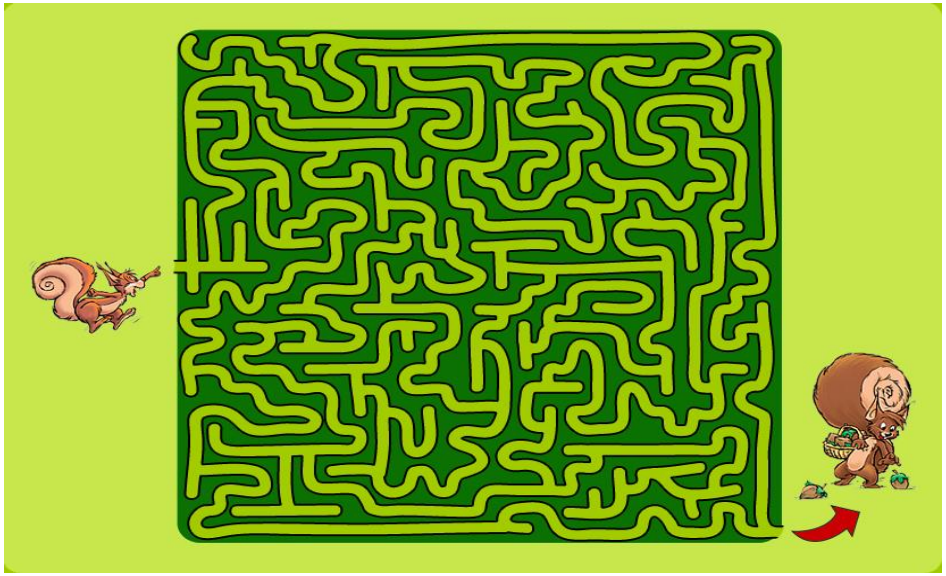
Molecular pathology

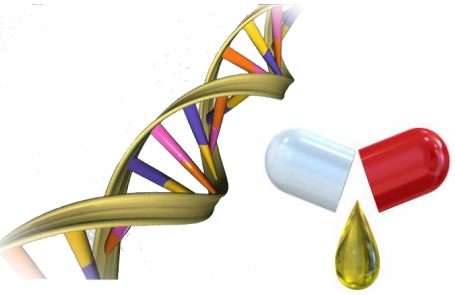
Molecular biology



This difference has a strong impact on the approach for accreditation procedure and to set up a validation method for molecular testing

The complexity of the Pre-Analytical steps in pathology





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"ISO 15189:2007 is for use by **medical laboratories** in developing their quality management systems and assessing their own competence, and for use by accreditation bodies in confirming or recognising the competence of medical laboratories"

International Organization for Standardization

- *Accreditation* [i.e., ISO 15189] is different from *Certification* [i.e., ISO 9001]
- Accreditation:
  - Quality Management System & Technical Competence
    - For patient health care
- Certification:
  - Quality Management System only
    - More adapted for research laboratories and biobanks

The ISO 15189:2007 referential

- **5 chapters=**
  - Chapter 1
    - Scope of application
  - Chapter 2
    - Normative references
  - Chapter 3
    - Terms and definitions
  - Chapter 4 **(+ 15 subchapters)**
    - Quality management system
  - Chapter 5 **(+ 8 subchapters)**
    - Technical requirements

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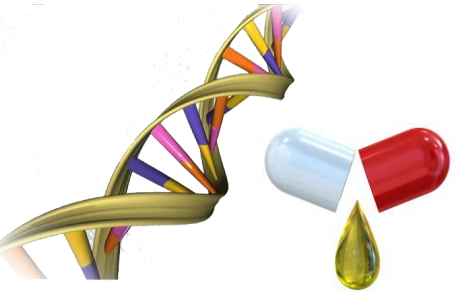

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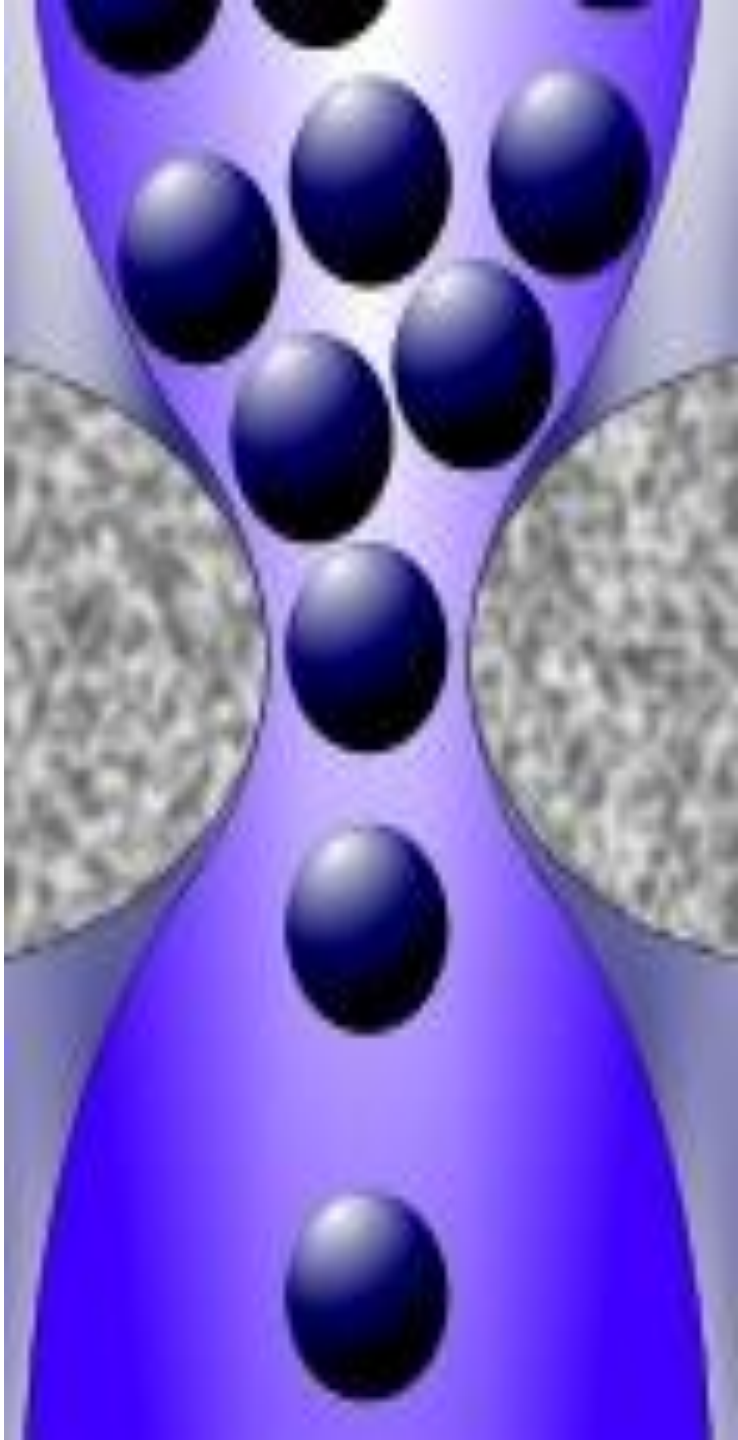
Bottlenecks*

- Pre analytical steps
- Constant working progress of technical solutions
- Constant changes in user needs
- Setting up the file of method validation
- Budget and investment
- Time consumming activity
- Great commitment and concentration of the whole team
- Example of pitfalls in molecular pathology

* This list is not exhaustive !



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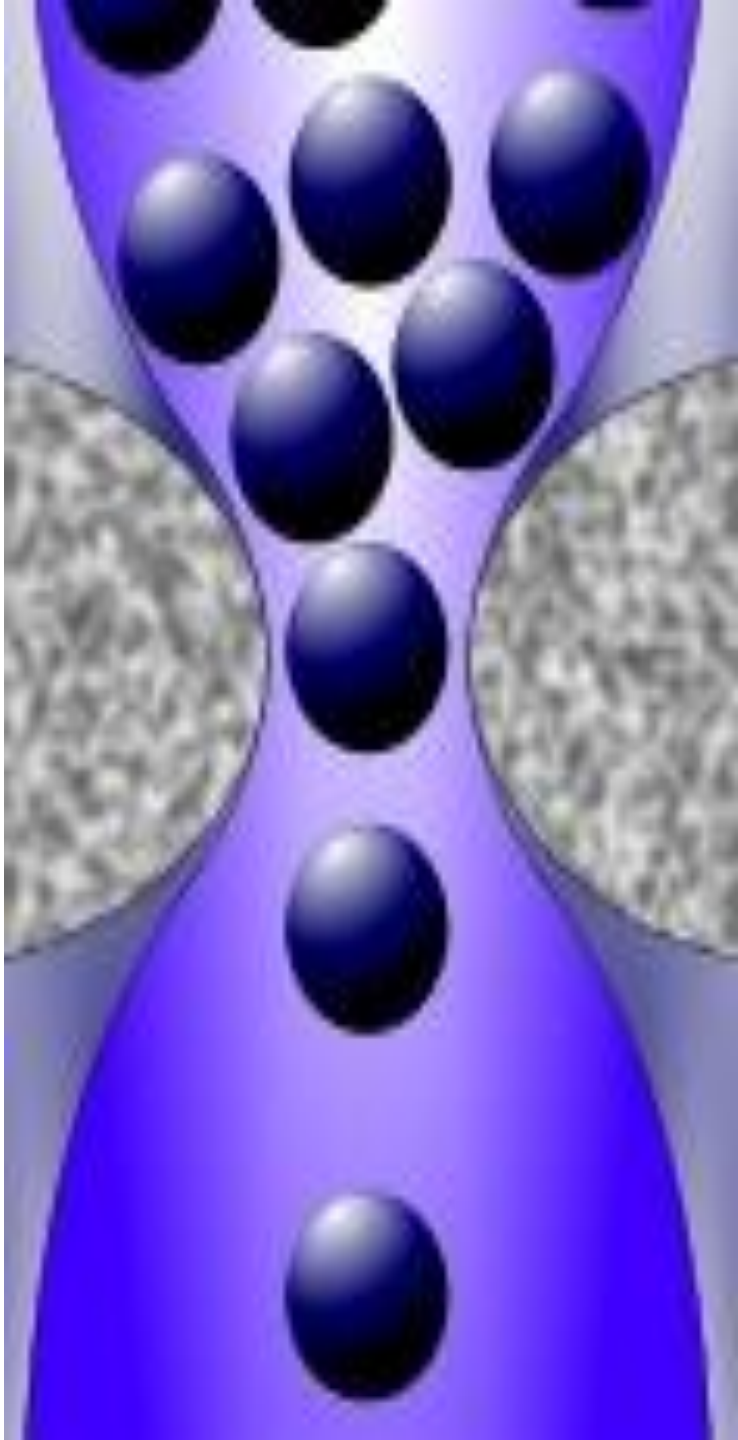
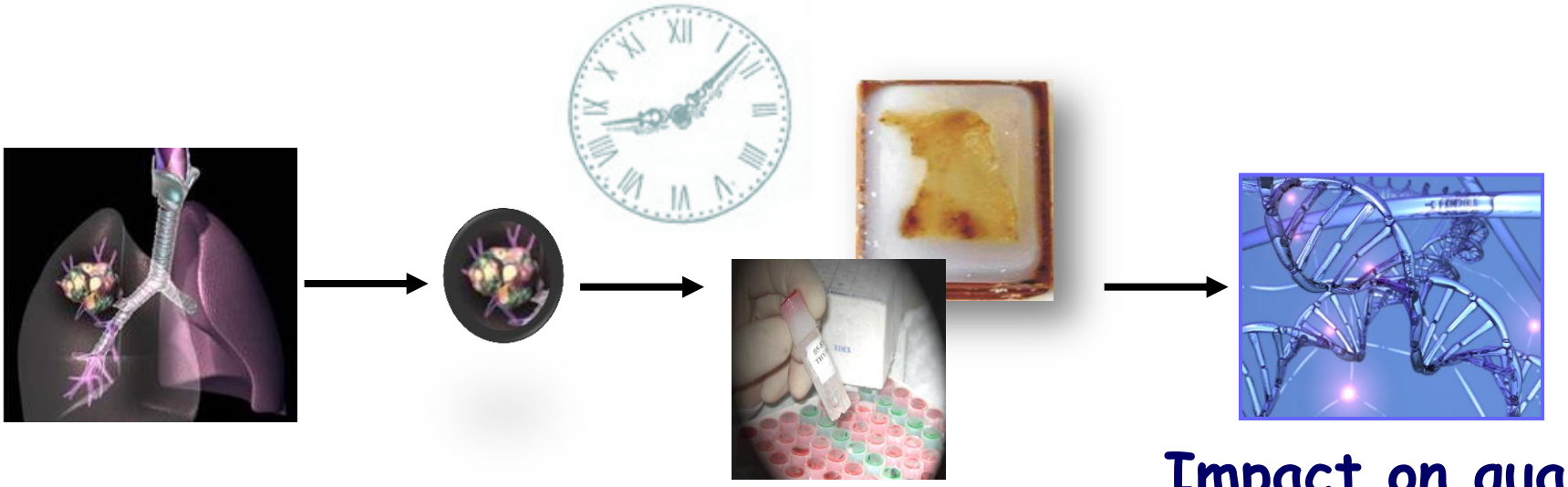


Molecular pathology

From frozen tissues and/or from fixed paraffin embedded tissues

How to control the different **P**re-**A**nalytical **T**ime **S**teps ?



Impact on quality

Physicians Pathology Lab Molecular Lab

Differences in tissue/cell preservation

Pre-Analytical Time Intervals

Surgery
Warm ischemia

Hospital
Organization
Cold ischemia

Transport to

Pathology department

Fixation

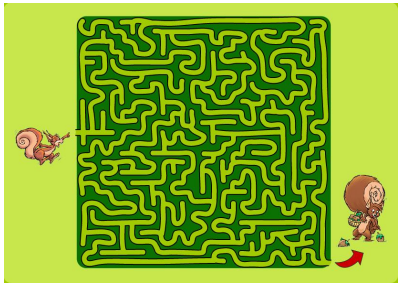
Grossing

Embedding

Archive

Formalin

Other fixatives



How to avoid a labyrinth ?

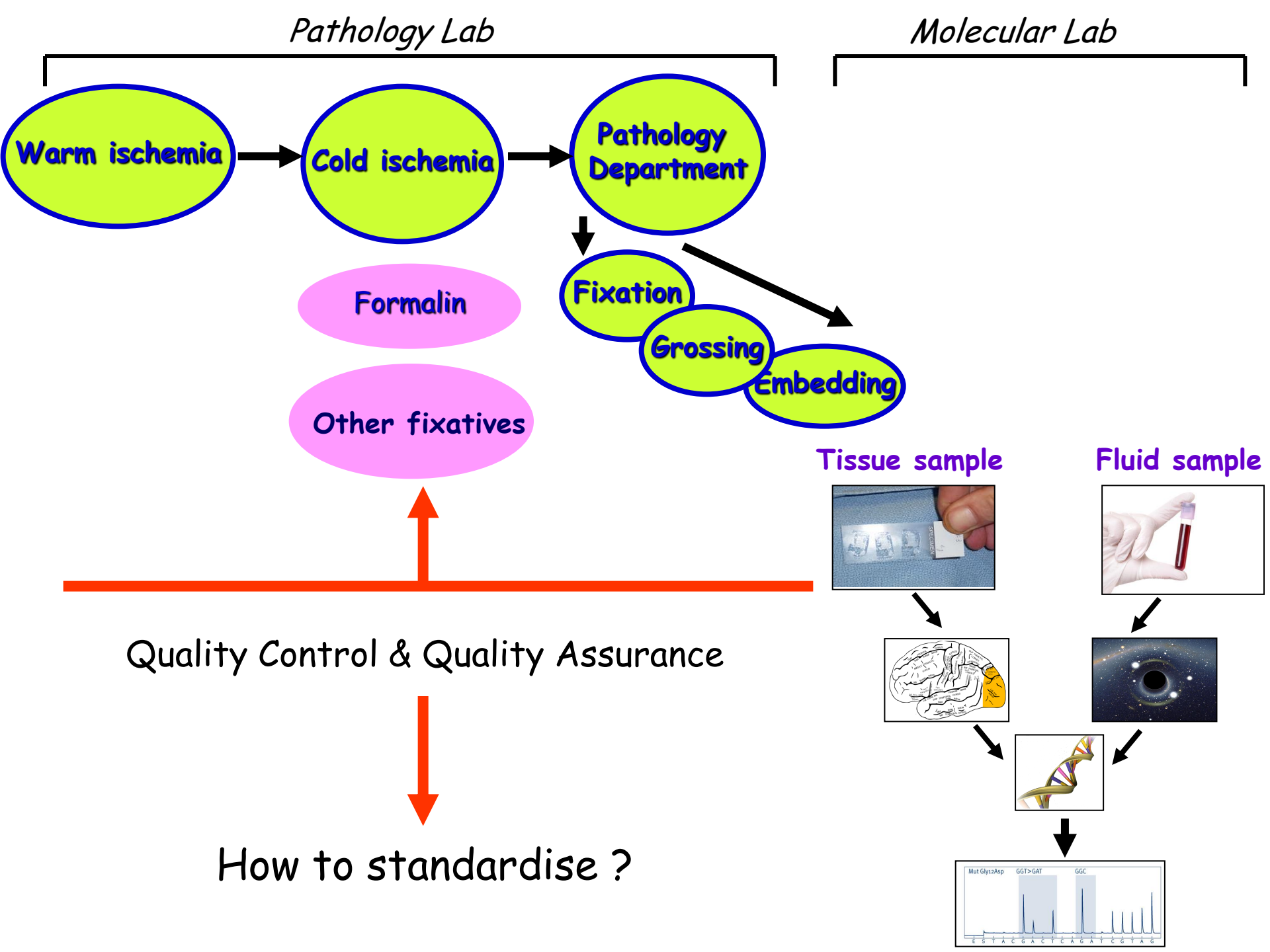
Sec-Hrs

Sec-Hrs-Days

Hrs-Days

Days-Years

Times


Pathology Lab
Molecular Lab
Warm ischemia
Cold ischemia
Pathology Department
Fixation
Grossing
Embedding
Formalin
Other fixatives
Tissue sample
Fluid sample
Quality Control & Quality Assurance
How to standardise ?

Working with…..

equipement and supplies….

Major bottlenecks

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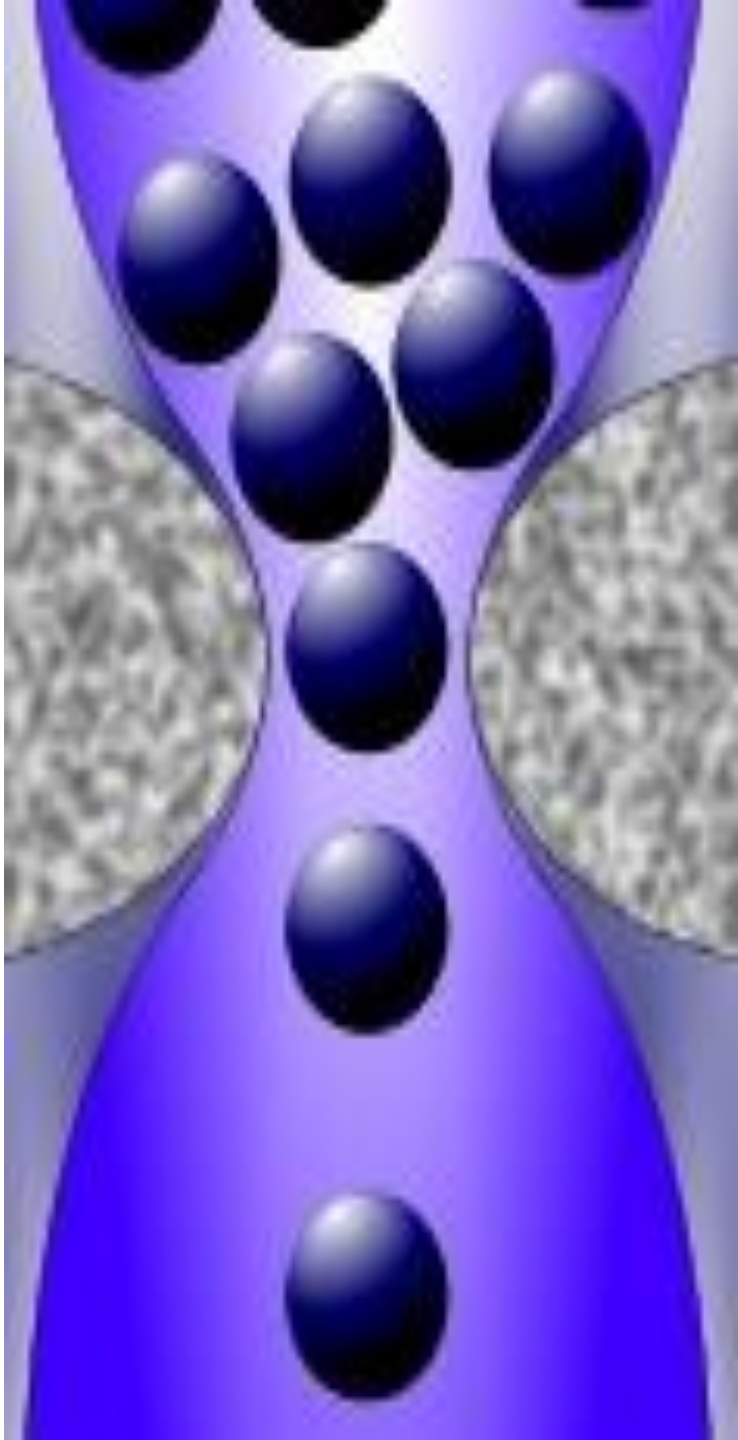
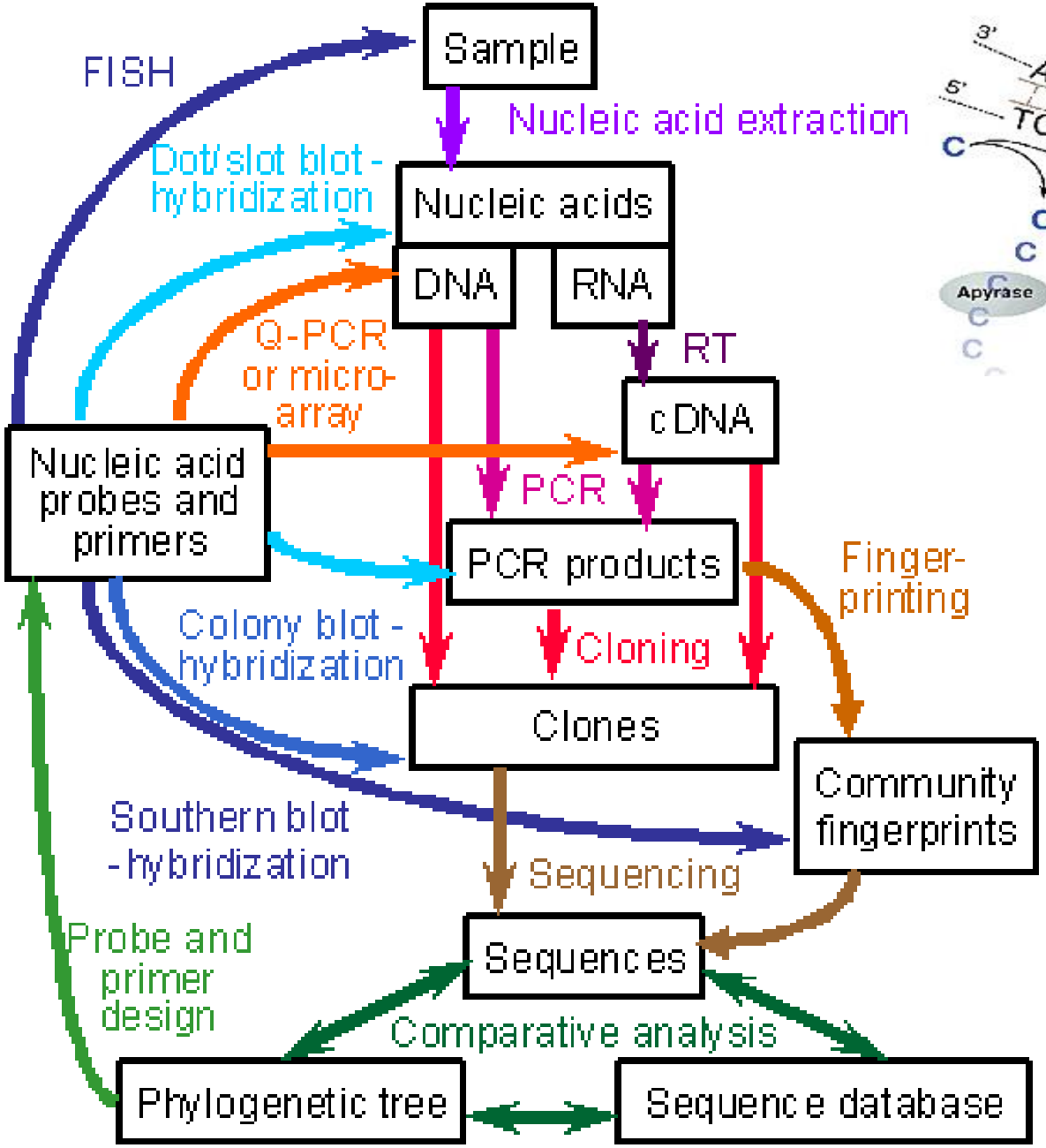
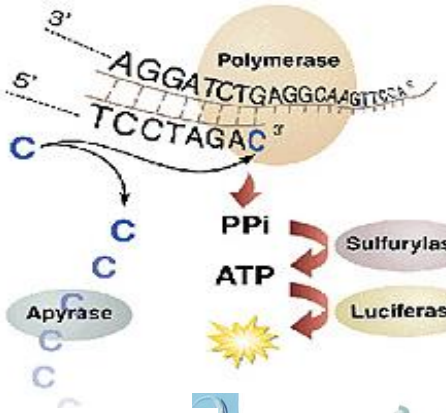
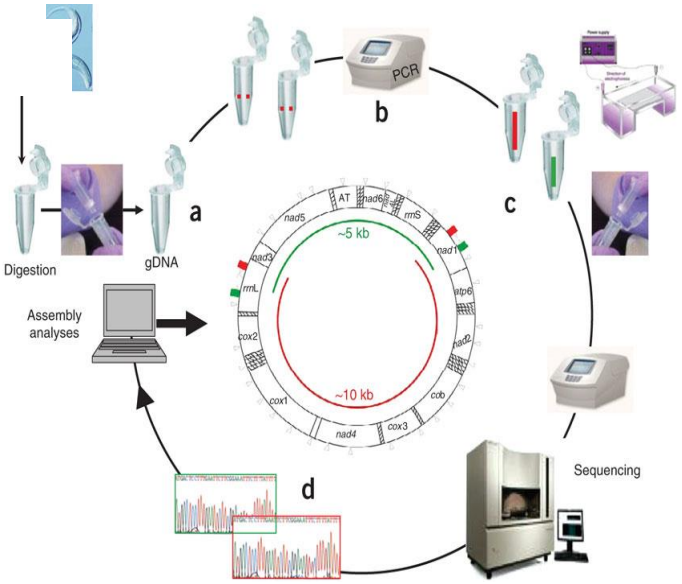
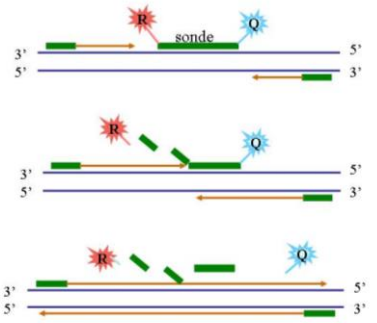
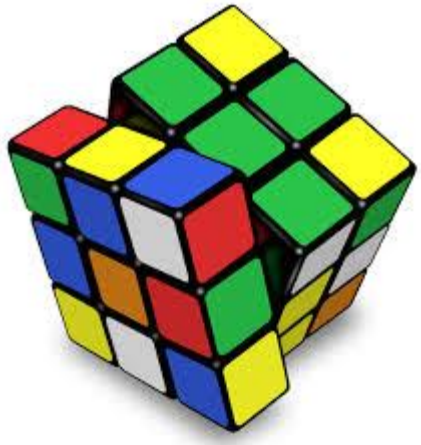



FISH
Sample
Nucleic acid extraction
Dot/slot blot - hybridization
Nucleic acids
DNA
RNA
Q-PCR or micro-array
RT
cDNA
Nucleic acid probes and primers
PCR
PCR products
Finger-printing
Colony blot - hybridization
Cloning
Clones
Community fingerprints
Southern blot - hybridization
Sequencing
Probe and primer design
Sequences
Comparative analysis
Phylogenetic tree
Sequence database


Polymerase
PPi
ATP
Sulfurylase
Luciferase
Apyrase


PCR
a
b
c
d
Digestion
gDNA
Assembly analyses
~5 kb
~10 kb
Sequencing


sonde



Do not change your technology approach during your process of accreditation !

This can easily become a headache !

An upgradable strategy ?

From a single gene target to several hundred of gene target

How to permanently make the modification of the different procedures setting up for an ISO 15189 accreditation ?

How to integrate the onset on the next generation sequencing technologies ?

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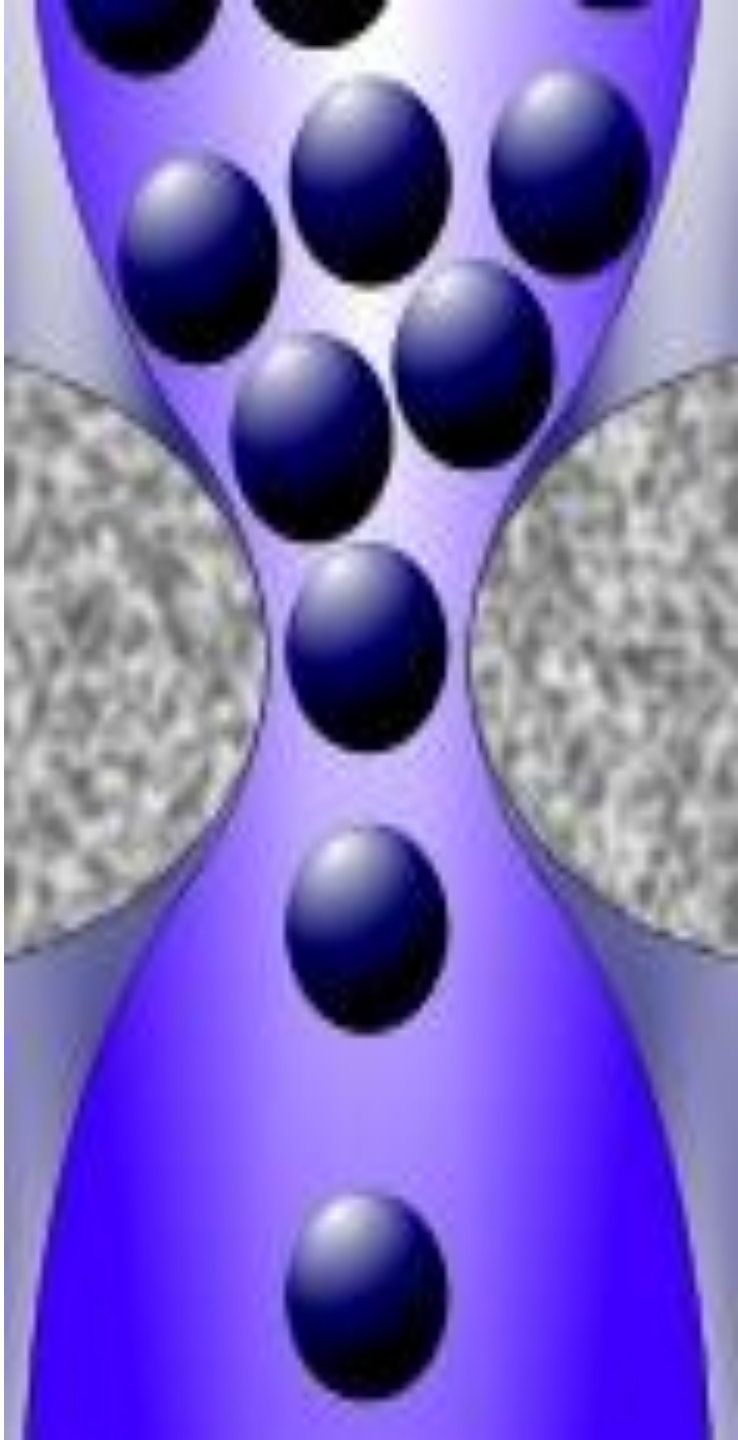
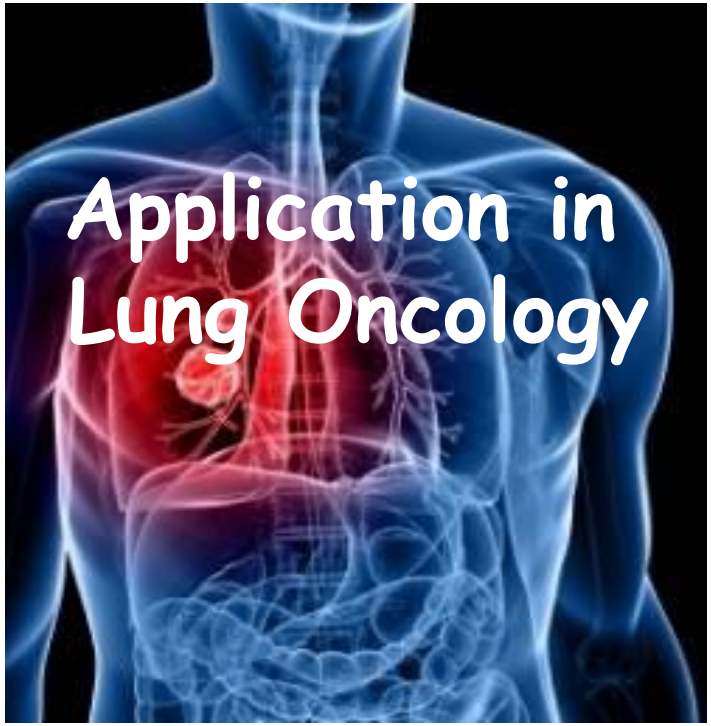


Lung cancer is the most prevalent cancer in the world

Lung cancer is the major cause of tumor-related mortality

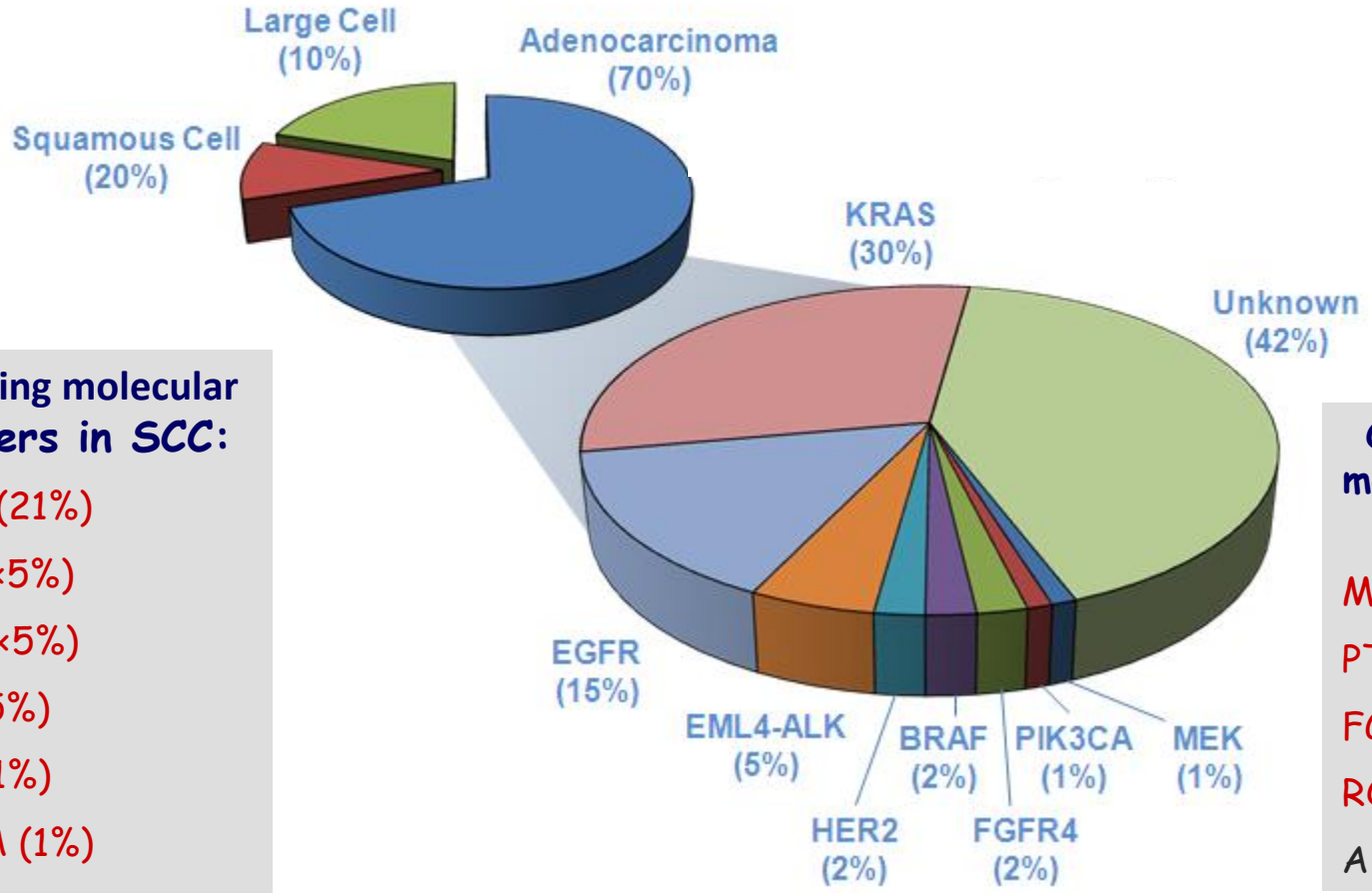
The cure rate of patients with lung cancer remains low (15% at 5 years)



Rapid emergence of numerous targeted therapies

Onset of primary and secondary drug resistances

Current estimate of the major druggable mutations in non small cell lung cancer



Emerging molecular markers in SCC:

FGFR1 (21%)

EGFR (<5%)

KRAS (<5%)

ALK (<5%)

AKT1 (1%)

PIK3CA (1%)

MET (2%)

HER2 (0.1%)

BRAF (0.1%)

MAP2K1 (0%)

Other emerging molecular markers in ADC:

MET (2%)

PTEN (1%)

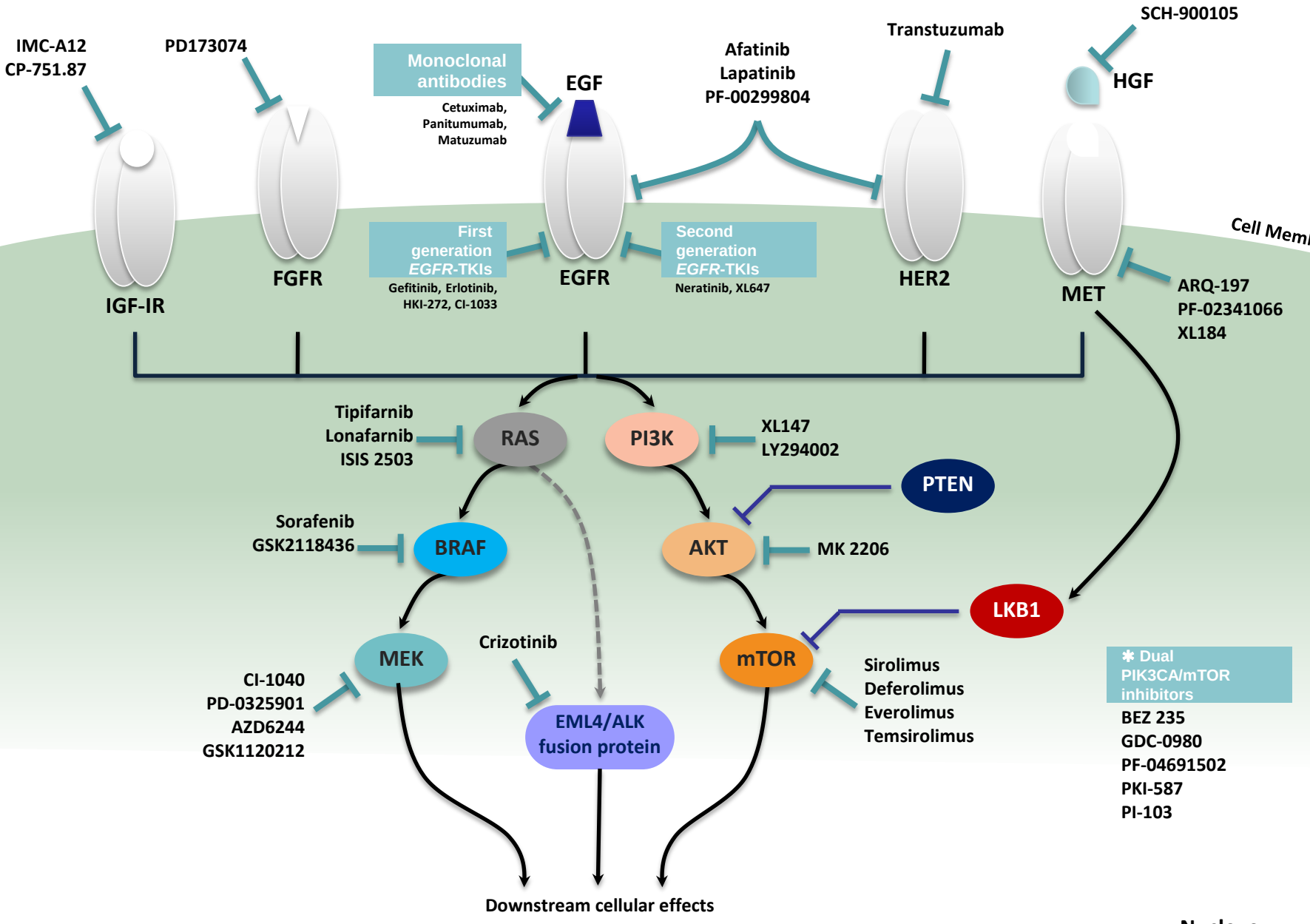
FGFR1 (2%)

ROS (0.5%)

AKT1 (0%)

P53 & LKB1: most frequent but non driving mutations!

A couple of target therapies in lung carcinoma !



Ilie M and Hofman P, CMC 2012

Oncologist needs

Rapid molecular pathology assessment

CLINICAL TRIALS

Experimental Treatment

May Be Right For You

And…. secondary resistances……

Major bottlenecks

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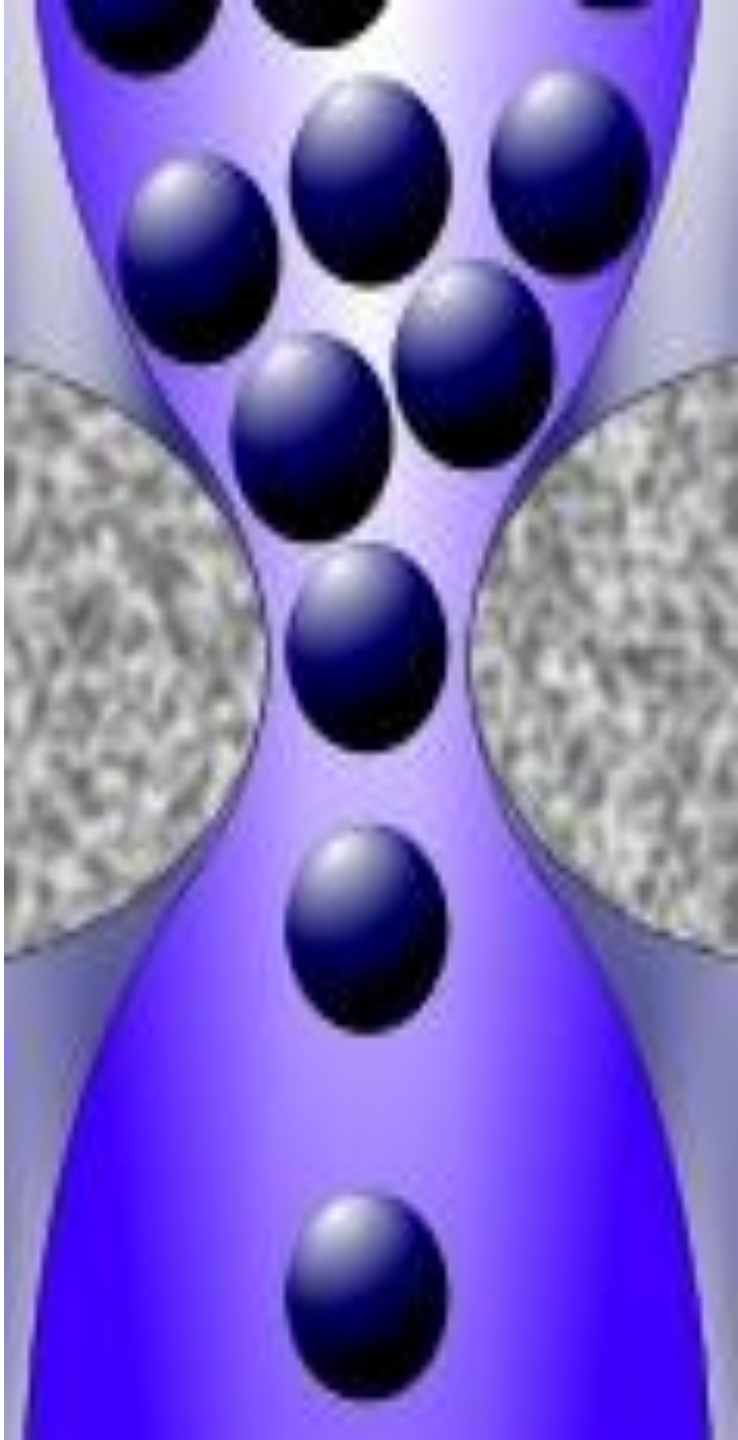

Issues to be considered when a new molecular diagnostic test is selected in the lab

1. Scientific literature evidence found in peer reviewed articles, guidelines, expert opinions.
2. The patient population for which the test will be designed has to be well defined since it might influence the other test selection criteria.
3. The selection of an adequate sample type has to take into account the ease of sample collection, the minimal sample volume needed, the recipient type (type of container, additives), the transport conditions in respect to the stability of the material, and the available literature evidence (peer review articles, guidelines).
4. The technique used for the examination procedure should be compatible with the required turn around time.
5. Practical consequences of the implementation of a new examination procedure in the current laboratory setting should be considered.
6. Technical and diagnostic test performances should meet the clinical needs. It is obvious that the opinion of the clinician requesting the examination procedure is of high value when the introduction of a new test is considered.
7. The total cost of the test should be calculated and should be in balance with the clinical and financial impact of the test result.

Validation file method

Major bottlenecks

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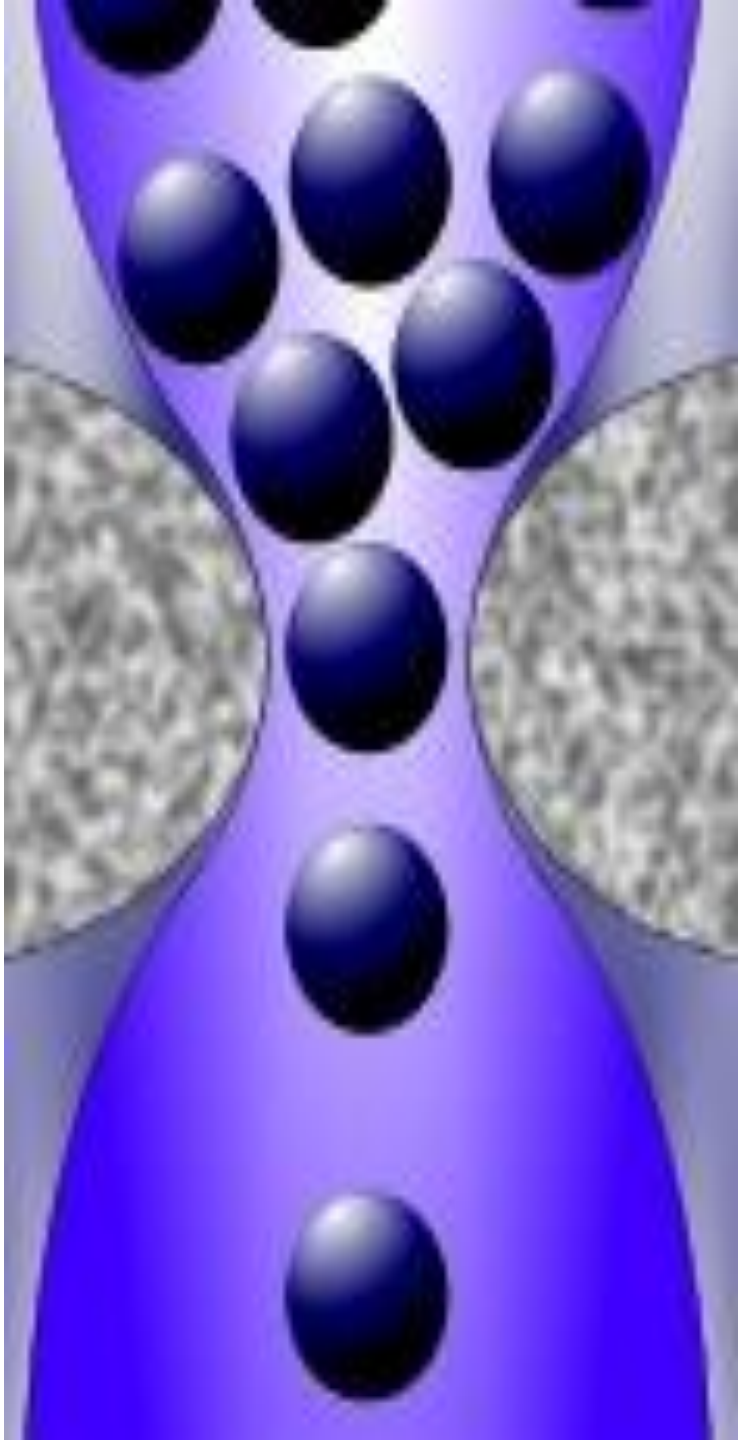

Estimated budget *in our institution* for the molecular laboratory accreditation (ISO 15189) project (2010-2012)

LPCE BIOBANK
Quality Management Tissue

300.000

Major bottlenecks

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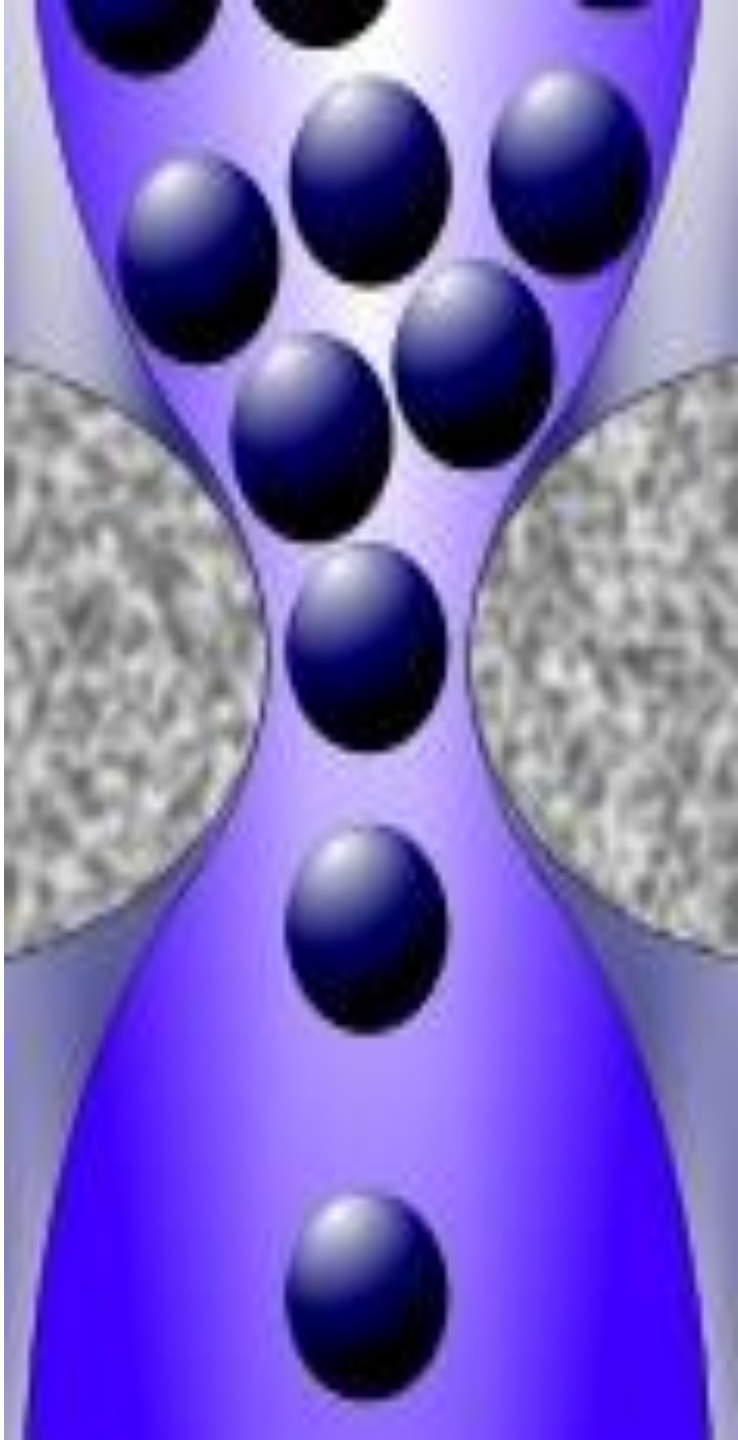

Estimated time period per week
for each technician of the lab

6 technicians: 5h x 4

Estimated time period per week
for each molecular pathologist of the lab

Three molecular pathologists: 8h x 3

LPCE BIOBANK
Quality Management Tissue

Full time period per week for
a dedicated ingenior

Major bottlenecks

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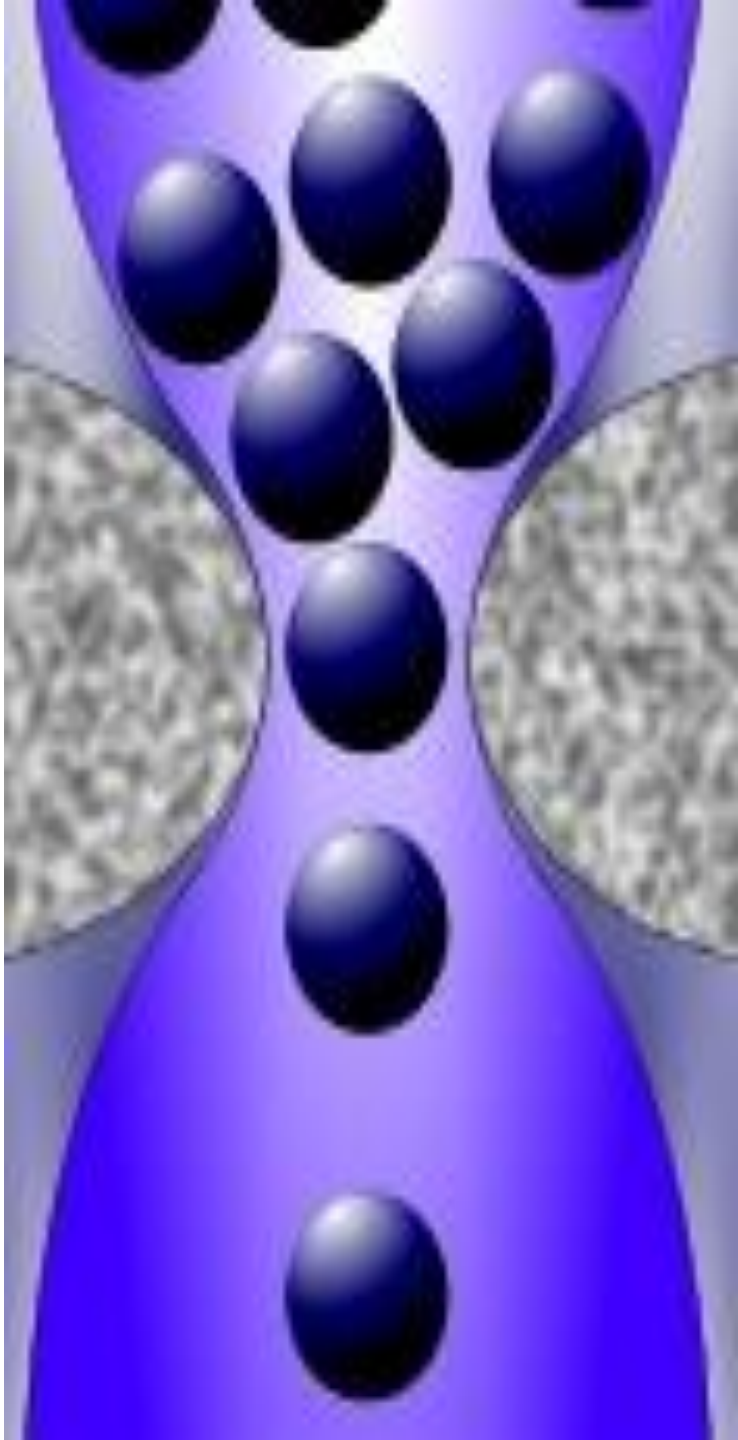
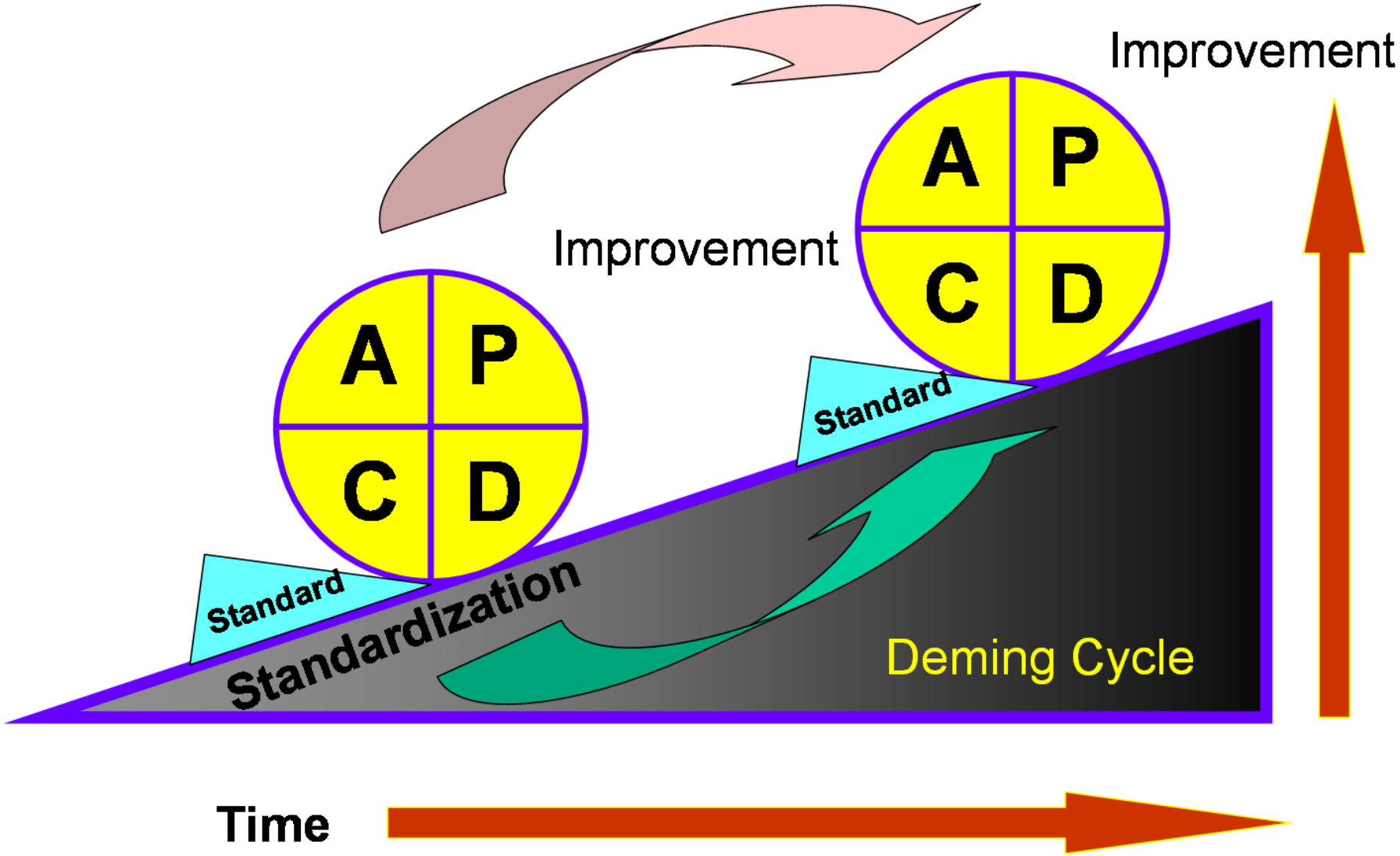


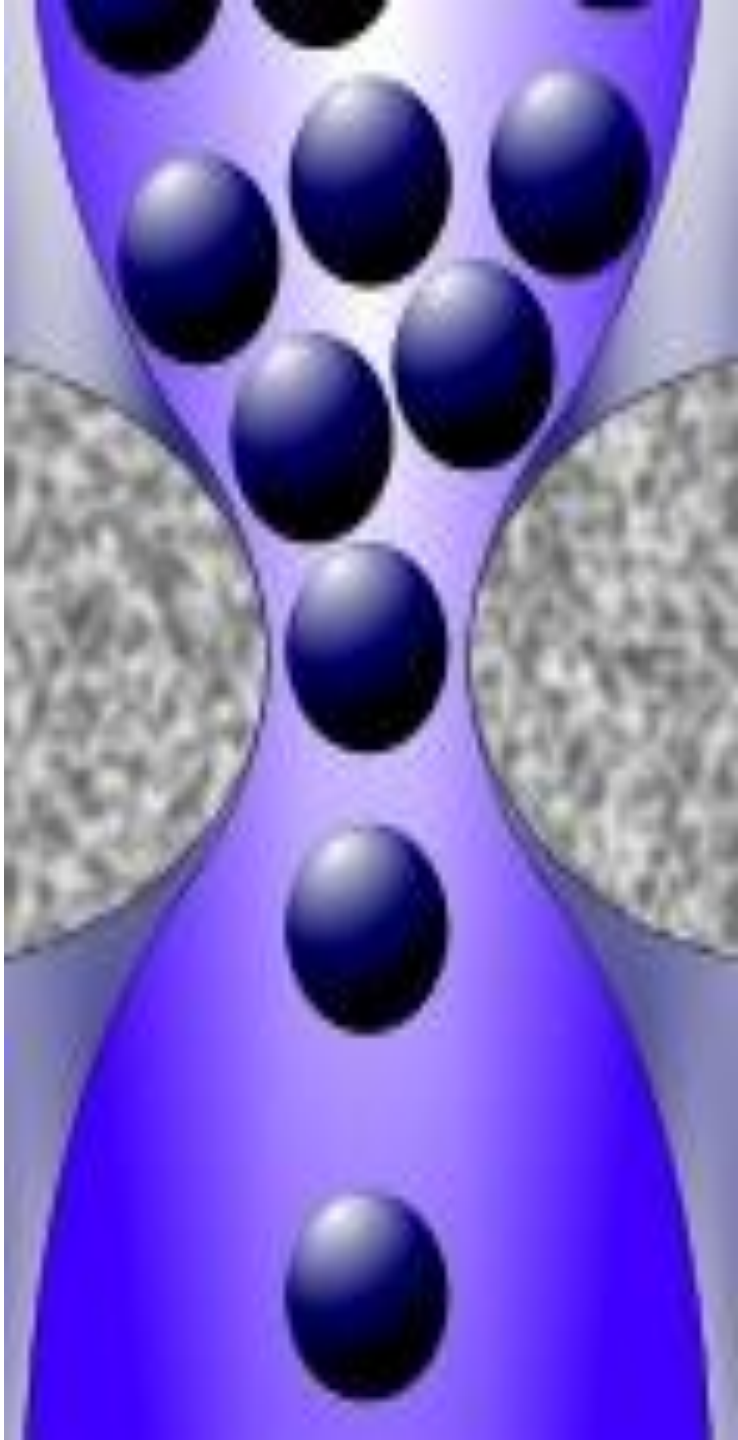
Inescapable !

Plan, Do, Check & Action



Major bottlenecks

- Pre analytical steps
- Constant working progress of technical solutions
- Constant changes in user needs
- Setting up the file of method validation
- Budget and investment
- Time consumming activity
- Require great commitment and concentration of the whole team
- Example of pitfalls in lung cancer molecular pathology



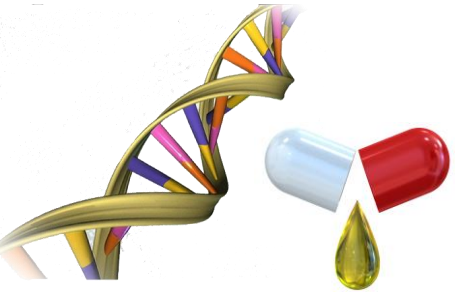
Clinical Pathology

Molecular Pathology

Table 1. Overview of Different Pitfalls and Recommendations at Each Step of Molecular Testing

Step	Pitfalls	Recommendation
Morphological sample assessment	Distinguish squamous cell carcinoma from adenocarcinoma and NSCLC NOS	Immunohistochemical (TTF1/p63 staining) and histochemical (mucin) studies May report "probably adenocarcinoma"
Material for molecular analysis	No knowledge of the percentage of tumor cells	Perform HE stain before molecular test
Time point	Extended tumaround time	At diagnosis or at disease progression if possible
Who may order?	Extended tumaround time	Treating physicians or pathologists
Sample quality	Warm ischemia time Cold ischemia time	Rapid transfer to the pathology laboratory (e.g., pneumatic air tube transport system)
Fixation	Type of fixative	10% NBF should be used. Bouin's or fixative substitutive fixatives should be avoided. Cryopreservation should be the standard method for tissue fixation and preservation
	Delay of fixation	Avoid prolonged fixation
Type of specimen	Limited size or available sample	Biopsy preferred to cytology specimens
Tumor content	Low percentage of tumor cells	Enrichment for tumor cells (macro- or microdissection)
	Minimum percentage of tumor cells required for molecular analysis	Adaptation according to the estimated analytical sensitivity of each method. Refusal if to low or absent.
Sample preparation	DNA extraction	Between 1 and 10 FFPE sections of 5- to 10-µm thickness. Avoid contamination (e.g., separate dedicated lab areas, changing blades, sample-to-sample traceability, regular cleaning and decontamination, dedicated sterile scalpel for dissection). Fresh-frozen material should be the standard. Interfering substances removal (e.g., melanin) Test DNA quality (Control PCR amplification) Optimized and controlled reagents.
	Analysis success rate	95% of samples with successful DNA extraction.
Analysis methods	Validated methods vs. "in-house" tests	Should validate and verify each method
	Screening vs. targeted methods	According to clinical needs in agreement with physicians
	Sensitivity	The lower detection limit should be set at 1% of tumor cells for highly sensitive methods and 25-30% for direct sequencing.
	Specificity	False-negative results may occur and can be avoided by regular external quality assessment controls. Mutations should always be independently confirmed in order to avoid false-positive results.
	Reduced performance of mutation immunohistochemistry	Needs further validation
	Interpretation of positive ALK rearrangement by FISH analysis	At least two experienced pathologists should perform the reading. Use positive and negative controls.
	Analysis success rate	97% of samples with correct mutation test results.
	Quality assurance	Needs accreditation / Guidelines / External Quality Assessment Programs
	Final report	Should be reported, in conjunction with the identification of patient and health care professional, the pathology diagnosis, details on the tissue block tested, sample source, sample size and quality, estimation of the proportion of tumor cells in the sample extracted for DNA amplification, the method used, estimated test sensitivity and specificity, test results (mutant or wild-type allele) and interpretation of results in the context of the indication for testing

Ilie M and Hofman P, CMC 2012



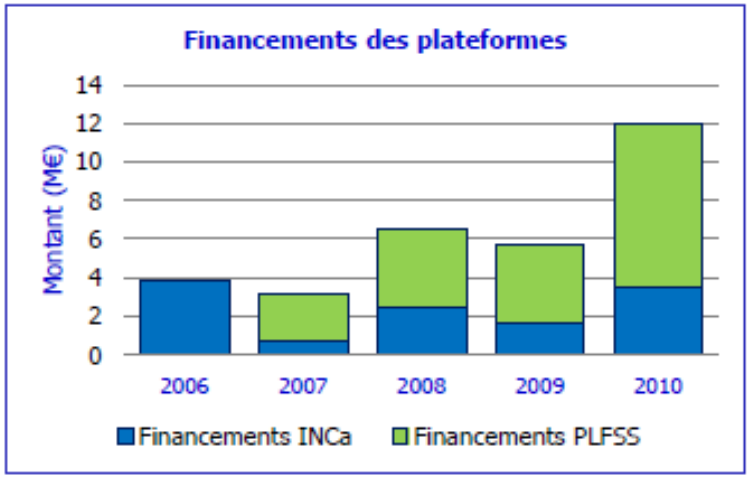
Agenda

- Main current challenges
- The times have changed: Molecular pathology is here to stay
- The ISO 15189:2007 standard
- Main bottlenecks and how to be successful ?
- The French organisation for molecular pathology testing in personalised medecine
- External Quality Control programme in France ?
- End-point

INSTITUT NATIONAL DU CANCER

28 Molecular Biology Platforms in France supported by the French National Cancer Institut (INCa)

(http://www.e-cancer.fr)



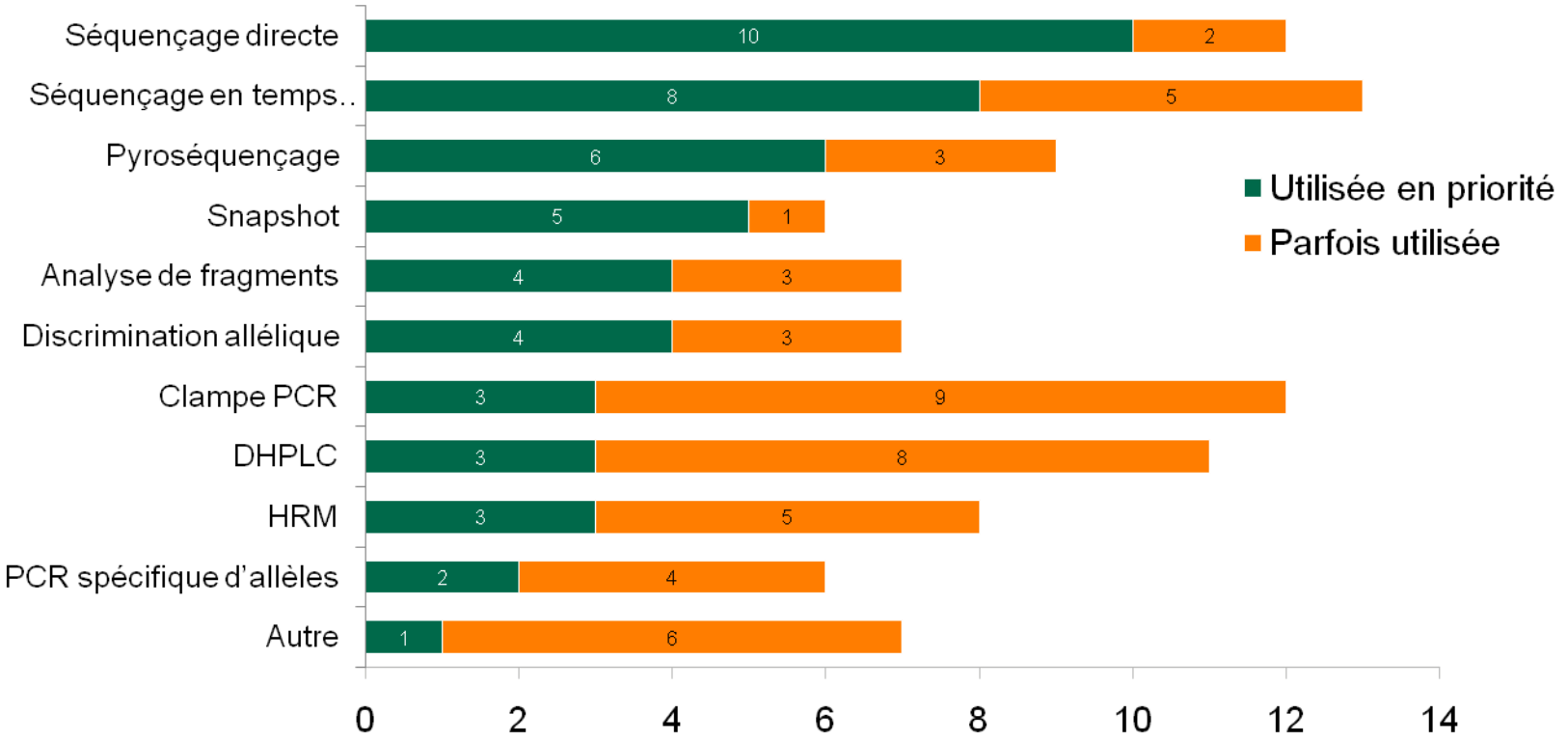
Grants from INCa

All patients in France (from private and public institutions) have access to molecular testing free of charge (*EGFR, KRAS, BRAF*, …)

LPCE BIOBANK
Quality Management Tissue

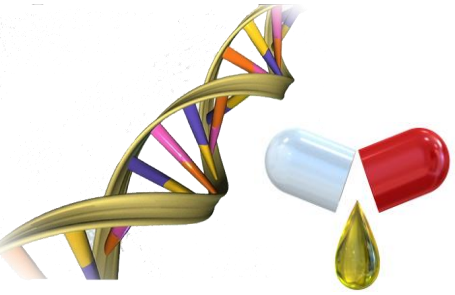
Methods used in the 28 French molecular pathology plateforms

Techniques utilisées



Take-away box

Taux de réponse: X% (Y centres)



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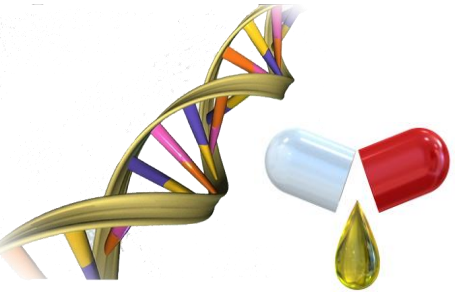
Different national EQC programmes

- 2011: *EGFR* mutation
- 2011: *KRAS* mutation
- 2012/2013: *BRAF* mutation; *KRAS* mutation; *EGFR* mutation
- Participation of 40 molecular pathology laboratories

All molecular pathology lab in France have to be accredited according to the ISO 15189 referential **in 2016**



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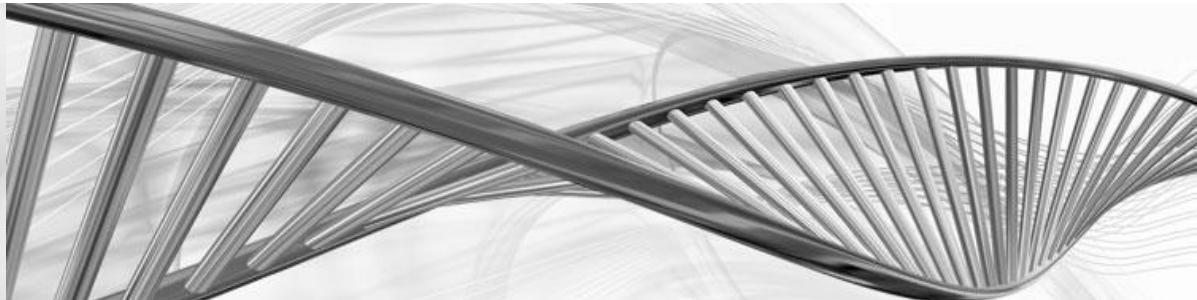
Accreditation for molecular pathology laboratories

- The introduction of targeted therapy into clinical oncology practice has created major opportunities in heath care area
- The integration of molecular diagnostics with therapeutics and the transition to personalised medecine are challenging !
- Among these challenges, getting the accreditation according to the ISO 15189 is the more critical point for a routine daily practice



Enjoy the ISO 15189 in a pathology molecular lab!

Getting an accreditation for molecular biology testing…..

…Is it a marshal baton ?

Getting an accreditation for molecular biology testing….

No….!

It is a clumber stick !

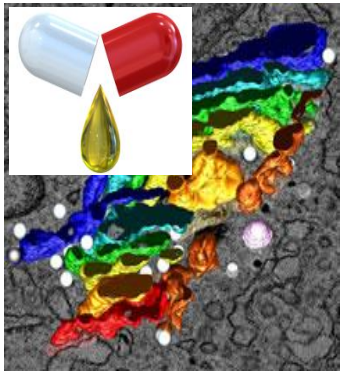
Create a virtual circle

Quality of health care service

Contribution to the good reputation of your lab

Financial Support !

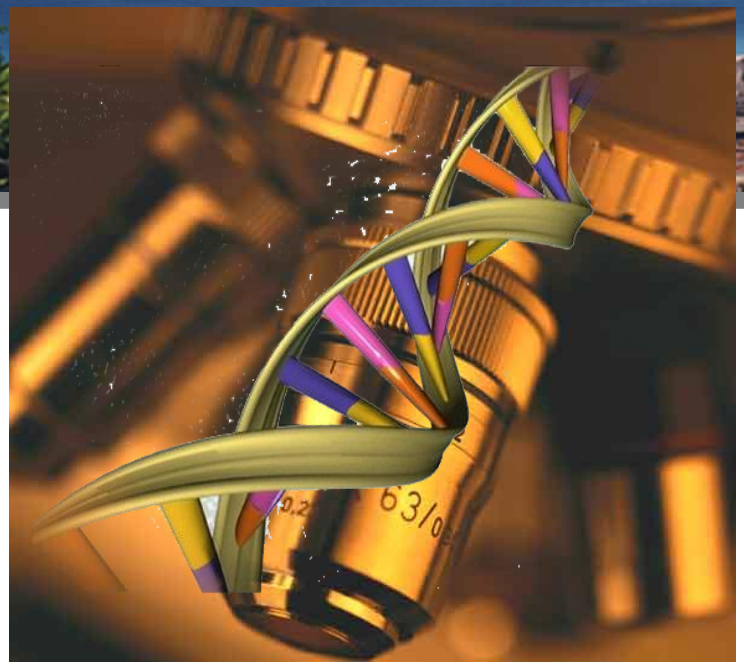
Accreditation



LPCE BIOBANK

Quality Management Tissue

CHU de Nice



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Breaking the silence