



Fondazione IRCCS  
Istituto Nazionale dei Tumori  
*via Venezian, 1 20133 Milano*

Sistema Socio Sanitario



Regione  
Lombardia

# ON BIOMARKER DATA QUALITY

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**Unit of Bioinformatic and Biostatistics**

**Fondazione IRCCS Istituto Nazionale dei Tumori di Milano**

**CHALLENGES IN DATA-DRIVEN GENOMIC COMPUTING**

**Organized by the Advanced ERC Project 693174 “GeCo” – data-driven Genomic Computing**

**Workshop in Como, Villa del Grumello – March 6-8 2019**

# Outline

## **I. Background**

- ✓ Biomarker definition
- ✓ Biomarker state of the art

## **II. Biomarker development process**

- ✓ Biomarker Discovery
- ✓ Biomarker Validation
- ✓ Clinical Utility

## **III. Concluding remarks**

The background is a light gray gradient. It features several realistic water droplets of various sizes, some with highlights and shadows, scattered across the surface. A faint, large, light gray circular pattern is also visible, centered behind the text.

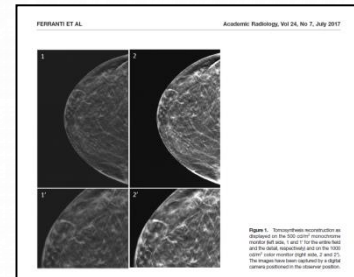
# **I. Background**

# What is a biomarker?

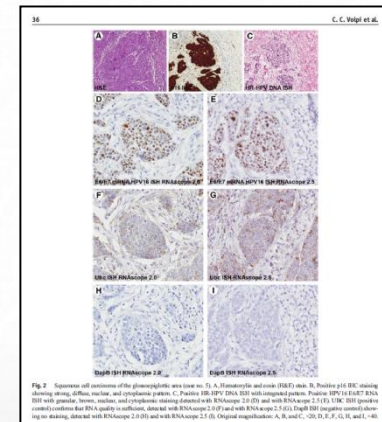
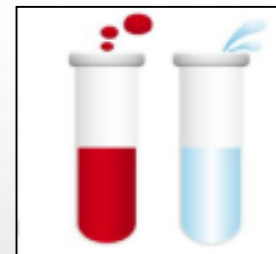
➤ a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.

- ✓ molecular characteristic
- ✓ histological characteristic
- ✓ radiographical characteristic
- ✓ physiological characteristic
- ✓ ....

types of biomarkers



Ferranti C, et al. Acad Radiol. 2017; 24:795–801



Volpi CC, et al. Hum pathology, 2017, 74:32-42.

# What is a tumor biomarker?

might be a:

- **molecular change** (i.e. nucleic acid, protein, or metabolite) OR
- **process change** (i.e. alteration in tissue or image appearance)

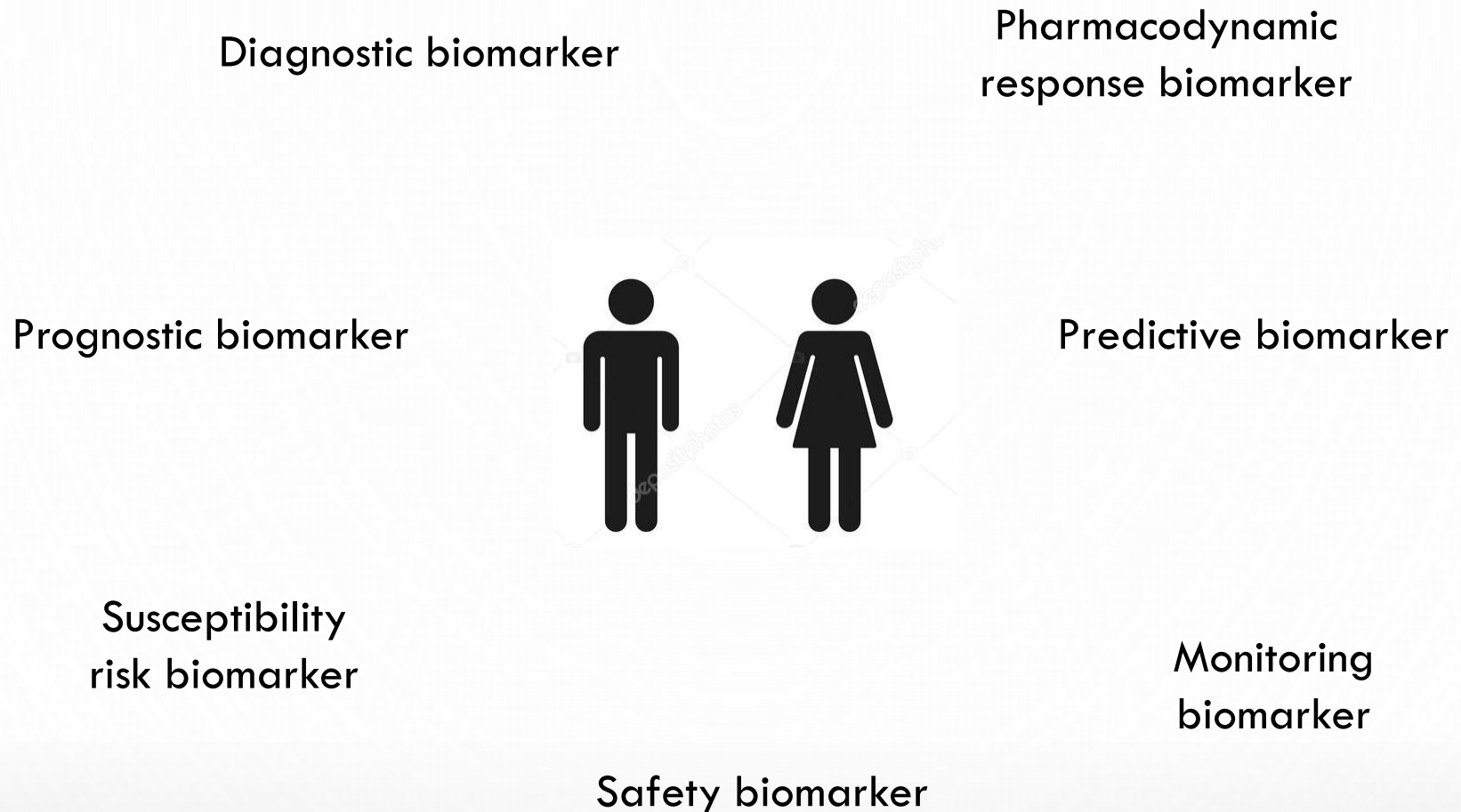
Can be detected in:

- ✓ tissue
- ✓ blood
- ✓ secretions (i.e. urine, stool, sputum, or breast nipple aspirates, CSF, etc)

# What is a biomarker test?

- ✓ a test used to identify or measure the perturbation reflected by the tumor biomarker
- ✓ must be applicable in clinical routine and reproducible and accurately translate the biomarker into a measurement parameter

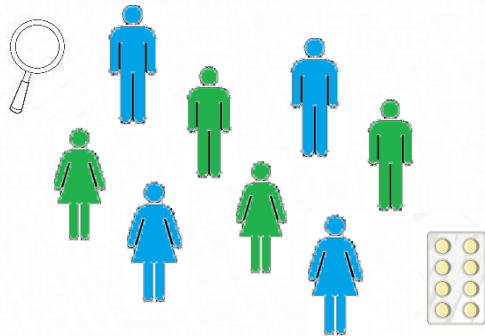
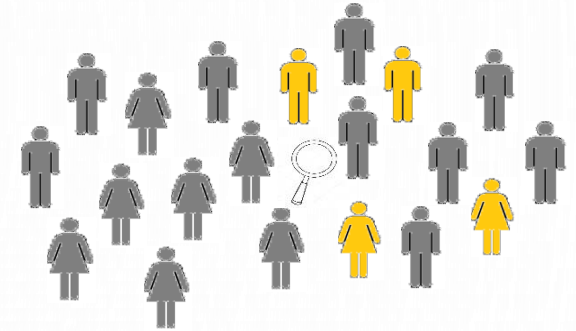
# Type of biomarker (1)





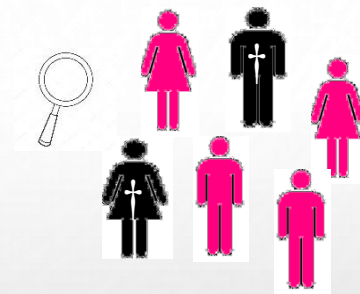
## Type of biomarker (2)

**Diagnostic**: “A biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease.”



**Predictive**: “A biomarker used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent.”

**Prognostic**: “A biomarker used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease or medical condition of interest.”





# Biomarkers state of the art (1)

PubMed Central® (PMC) is a free full-text archive of biomedical and life sciences journal literature at the U.S. National Institutes of Health's National Library of Medicine (NIH/NLM) (<https://www.ncbi.nlm.nih.gov/pmc/>)

The screenshot shows the PubMed website interface. At the top, the URL is [www.ncbi.nlm.nih.gov/pubmed/](http://www.ncbi.nlm.nih.gov/pubmed/). The header includes the NCBI logo, "Resources" and "How To" links, and a "Sign in to NCBI" button. Below the header, the "PubMed.gov" logo is displayed, along with a search bar containing the term "biomarkers" and a "Search" button. The search results are displayed in a table format. The first result is titled "Best matches for 'biomarkers':". Below this, three articles are listed: "The role of novel biomarkers in predicting diabetic nephropathy: a review" by Uwaezuoke SN et al. (Int J Nephrol Renovasc Dis. (2017)), "Novel Biomarkers of Heart Failure" by Savic-Radojevic A et al. (Adv Clin Chem. (2017)), and "Osteoarthritis biomarkers: year in review" by Watt FE et al. (Osteoarthritis Cartilage. (2018)). A button labeled "Switch to our new best match sort order" is visible. On the right side, there are filters for "Sort by" (Best match, Most recent) and "Results by year". Below these, there are sections for "Titles with your search terms" and "Multiple Epigenetic Biomarkers for evaluation of Students' Academic P [Genes Brain Behav. 2019]". At the bottom, the search results are summarized as "Items: 1 to 20 of 463055".

huge number of research studies focused on biomarkers in  
many different field and specimen

# Biomarkers state of the art (2)

The screenshot shows the PubMed website interface. The search bar contains the query "cancer biomarkers" OR "tumor biomarkers". The search results are displayed in a list format. The first result is "Liquid biopsy in ovarian cancer: recent advances on circulating tumor cells and circulating tumor DNA" by Giannopoulos L et al. (2018). The second result is "Lung Cancer Biomarkers" by I H et al. (2015). The third result is "Validation of new cancer biomarkers: a position statement from the European group on tumor markers" by Duffy MJ et al. (2015). The search results are sorted by "Most recent" and there are 131337 items found. A red circle highlights the "Search results" section. The "Items: 1 to 20 of 131337" text is also visible.

www.ncbi.nlm.nih.gov/pubmed/

NCBI Resources How To Sign in to NCBI

PubMed "cancer biomarkers" OR "tumor biomarkers" Search

US National Library of Medicine National Institutes of Health

Create RSS Create alert Advanced Help

Article types  
Clinical Trial  
Review  
Customize ...

Text availability  
Abstract  
Free full text  
Full text

Publication dates  
5 years  
10 years  
Custom range...

Species  
Humans  
Other Animals

Clear all  
Show additional filters

Format: Summary Sort by: Most Recent Per page: 20 Send to Filters: Manage Filters

Best matches for "cancer biomarkers" OR "tumor biomarkers":

[Liquid biopsy in ovarian cancer: recent advances on circulating tumor cells and circulating tumor DNA](#)  
Giannopoulos L et al. Clin Chem Lab Med. (2018)

[Lung Cancer Biomarkers](#)  
I H et al. Adv Clin Chem. (2015)

[Validation of new cancer biomarkers: a position statement from the European group on tumor markers](#)  
Duffy MJ et al. Clin Chem. (2015)

Switch to our new best match sort order

Sort by:  
Best match Most recent

Results by year

Download CSV

Titles with your search terms

Integration of metabolomic and transcriptomic profiles to identify biomarkers [J Cell Biochem. 2019]

Contribution of Serum Biomarkers to Prognostic

Search results  
Items: 1 to 20 of 131337

<< First < Prev Page 1 of 6567 Next > Last >>

~30% are focused "oncological" biomarkers

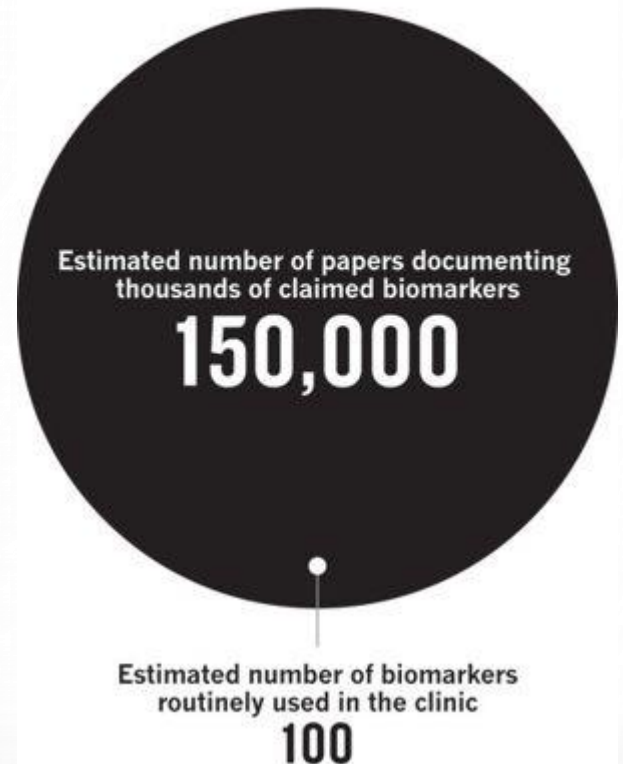
# However....

- ❑ a limited number of biomarkers is really translated and fully applied in the real clinical practice
- ❑ very few discovered biomarkers are used in the routine clinical setting

## Why?

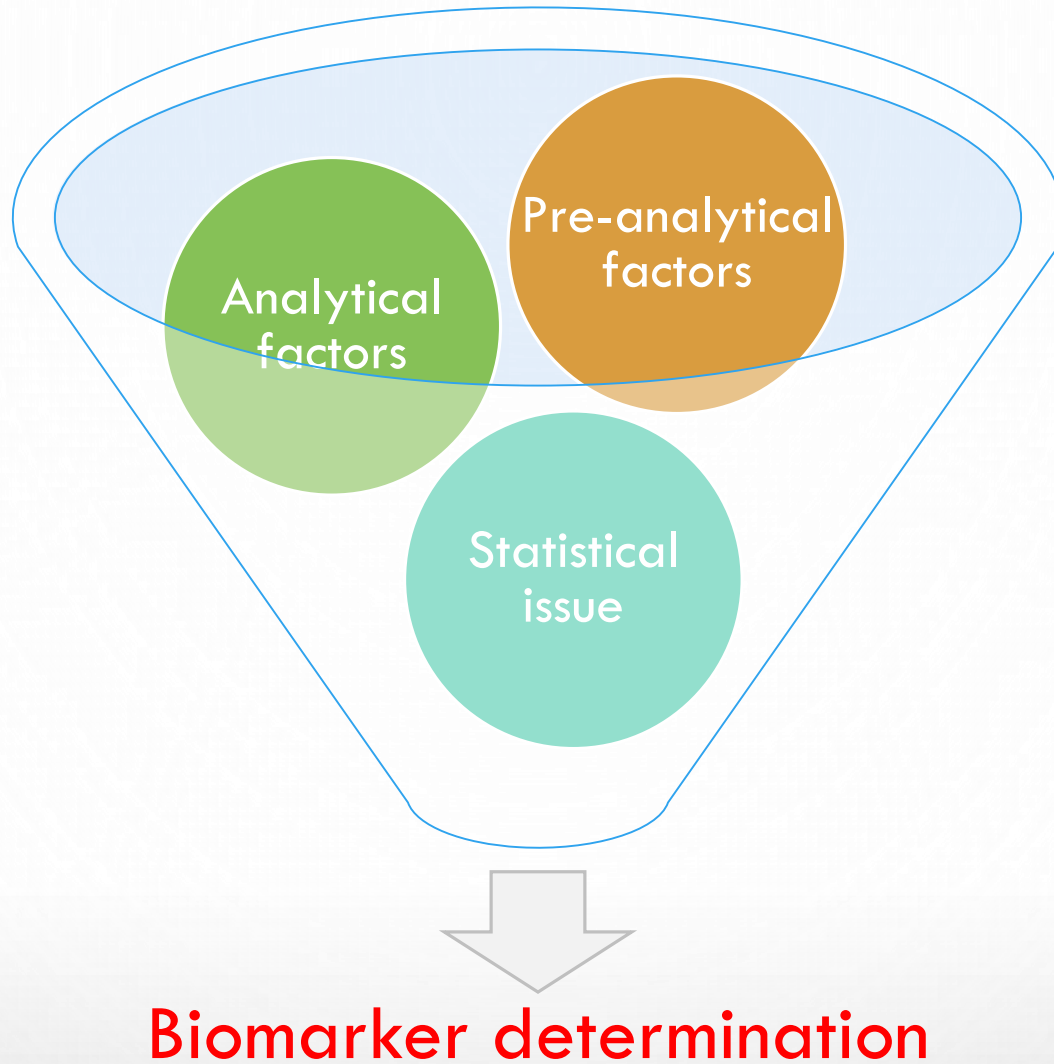
### A DROP IN THE OCEAN

Few of the numerous biomarkers so far discovered have made it to the clinic.



Poste G. Nature 2011;469:156-157

# Challenges in biomarker development (1)



# Challenges in biomarker development (2)

## Pre-analytical factors

- ✓ starting material
- ✓ sample collection and processing
- ✓ sample storage conditions
- ✓ type of extraction method
- ✓ ...

## Analytical factors

- ✓ type of detection method
- ✓ type of throughput
- ✓ type of commercial platform
- ✓ ...

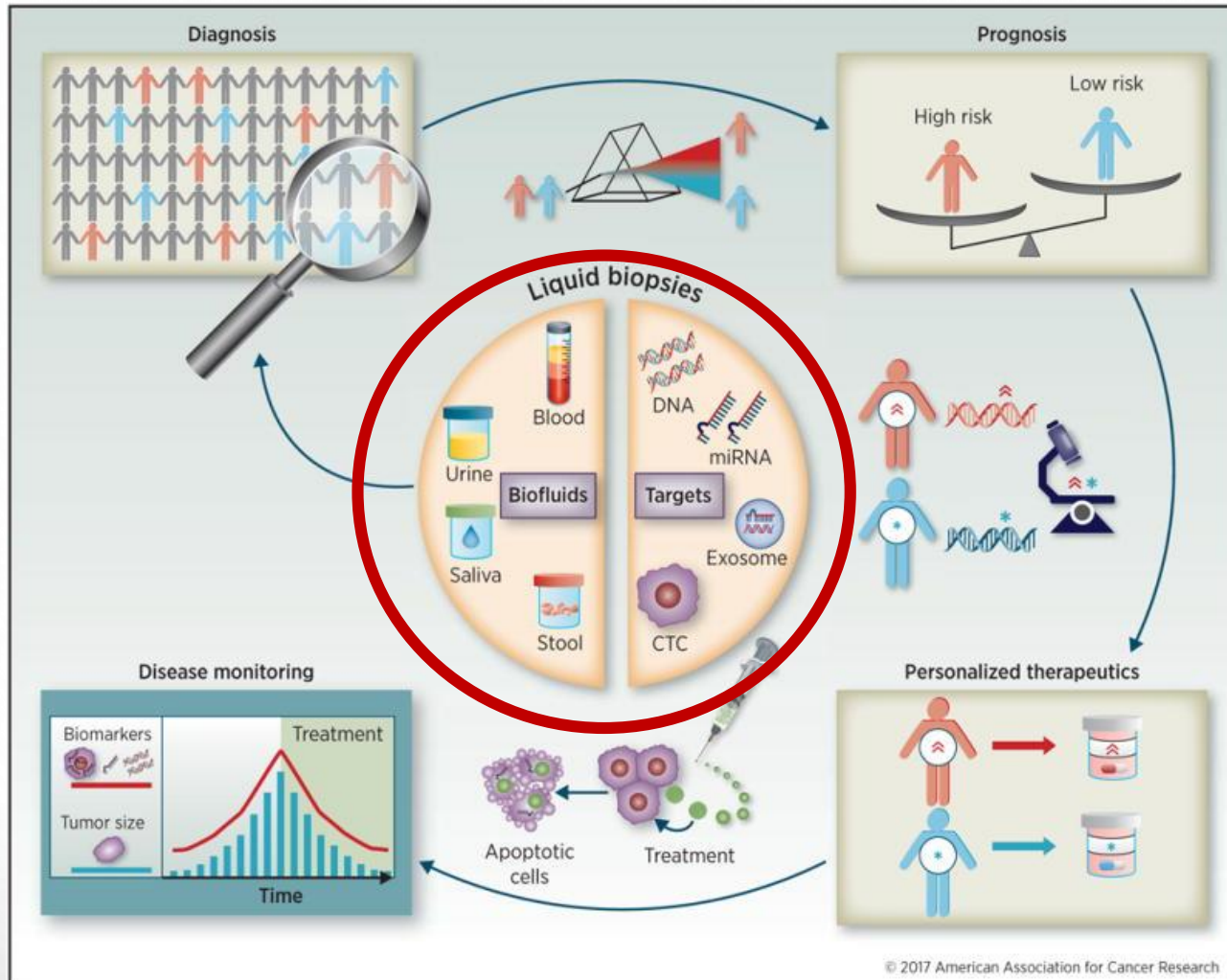
## Statistical analysis

- ✓ data normalization
- ✓ level and type of analysis
- ✓ type of predictor
- ✓ methods for multivariate model building
- ✓ ...

# Challenges in biomarker development (3)

- ❑ Molecular features measurable from **body fluids (i.e. Liquid biopsies)**
  - a great opportunity to develop non invasive biomarkers
  
- ❑ Availability of **high-throughput technologies**
  - new diagnostic and therapeutic perspective especially in the oncological scenario

# Challenges in biomarker development (3)



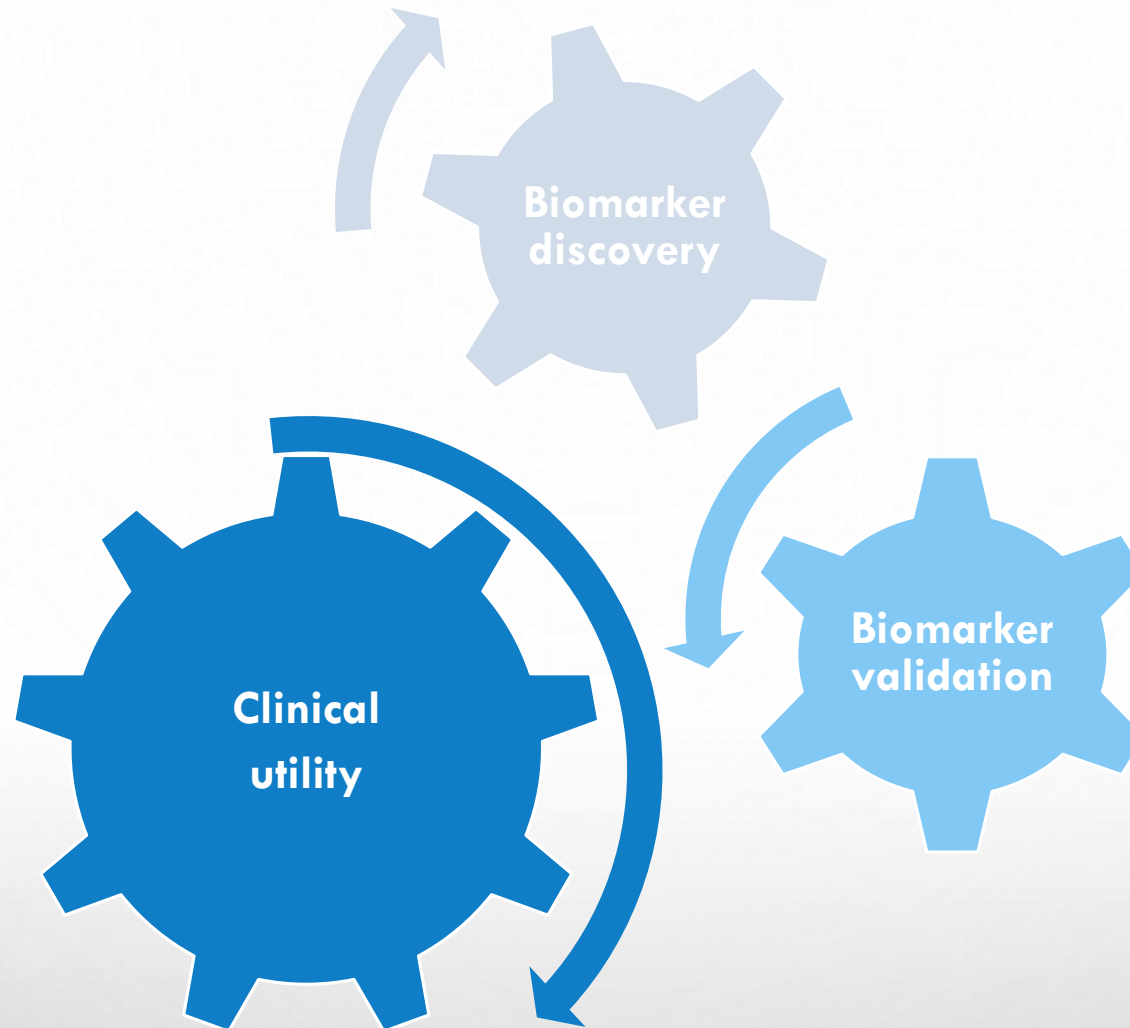
Shigeyasu K, Toden S, Zumwalt TJ, Okugawa Y, Goel A. Clin Cancer Res. 2017;23(10):2391-2399.



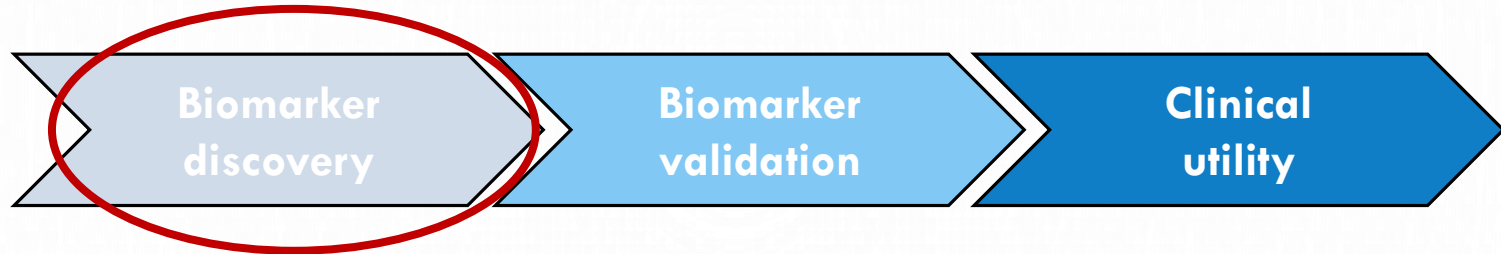
The background of the slide is a light gray gradient. It is decorated with numerous realistic water droplets of various sizes. Some droplets are large and prominent, while others are small and subtle. They are scattered across the slide, with a higher concentration in the top-left and bottom-right corners, and a few in the center. Each droplet has a soft shadow and a highlight, giving it a three-dimensional appearance.

## **II. Biomarker development process**

# Comprehensive evaluation of biomarker development process



# Biomarker discovery (1)



## 1. **identification of the context of use [study planning]**

- the fundamental use of the biomarker
- the target population (with inclusion and exclusion criteria)
- specific clinical context
- ...

## 2. **setting-up of the conditions for experiment running [data generation]**

- sample specimen and collection (e.g. collection tubes, timing of collection)
- sample processing (e.g. timing of processing, temperature of storage)
- sample analysis (e.g. type of extraction, platform)
- ...

## 3. **biomarker identification/selection [data analysis]**

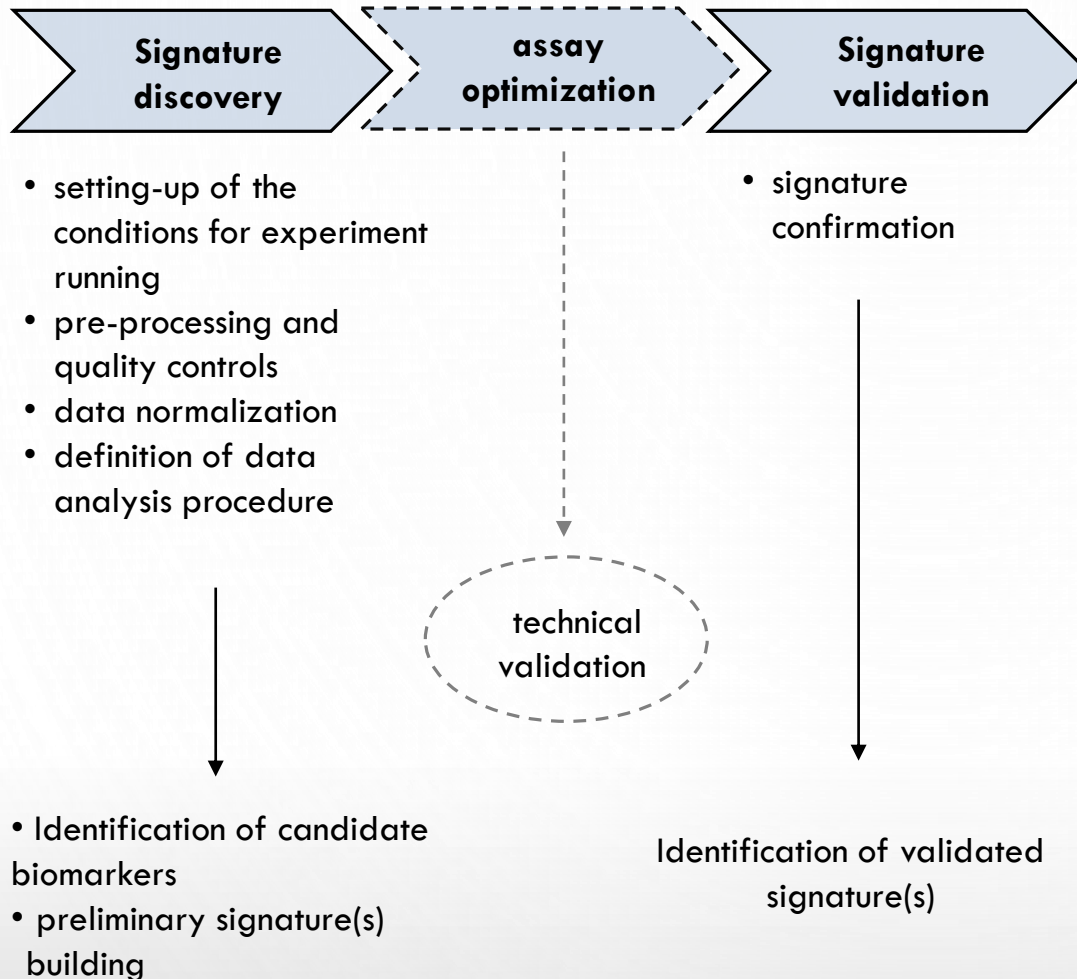
- pre-processing and quality controls
- definition of data analysis procedure
- identification of candidate biomarker
- development of omics-based signatures (e.g. statistical model)
- ...

# Biomarker discovery (2)

laboratory

clinic

## omics-based signature generation



# Biomarker discovery (3)

laboratory

clinic

omics-based signature generation



**Validated omics-signature(s) =  
biomarkers**

The screenshot shows the homepage of the AACR Clinical Cancer Research journal. The header includes the AACR logo and navigation links. The main content area features the journal title 'Clinical Cancer Research' and a search bar. Below the title, there is a navigation menu with links to Home, About, Articles, For Authors, Alerts, and News. The featured research article is titled 'Plasma microRNA levels for predicting therapeutic response to neoadjuvant treatment in HER2-positive breast cancer: Results from the NeoALTTO trial' by Serena Di Cosimo et al. The article's DOI is 10.1158/1078-0432.CCR-18-2507.

AACR Publications ▼ AACR American Association for Cancer Research

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## Clinical Cancer Research

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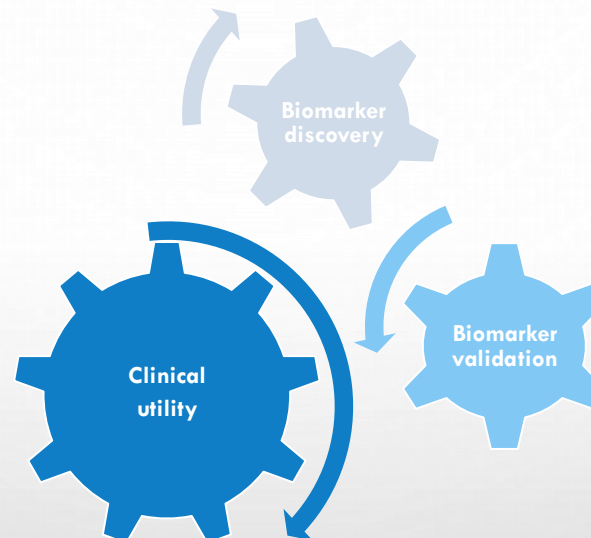
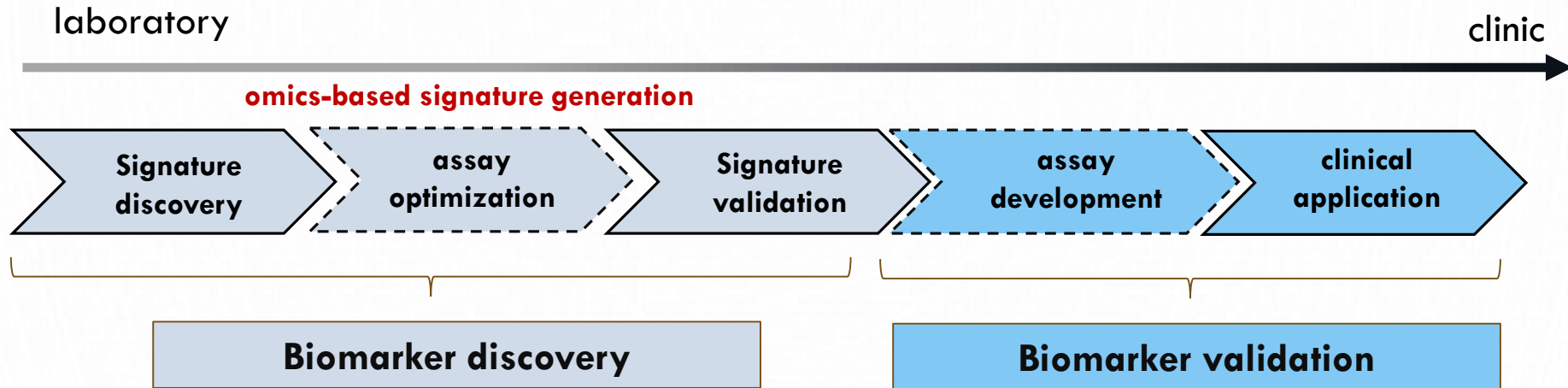
Research Article

### Plasma microRNA levels for predicting therapeutic response to neoadjuvant treatment in HER2-positive breast cancer: Results from the NeoALTTO trial

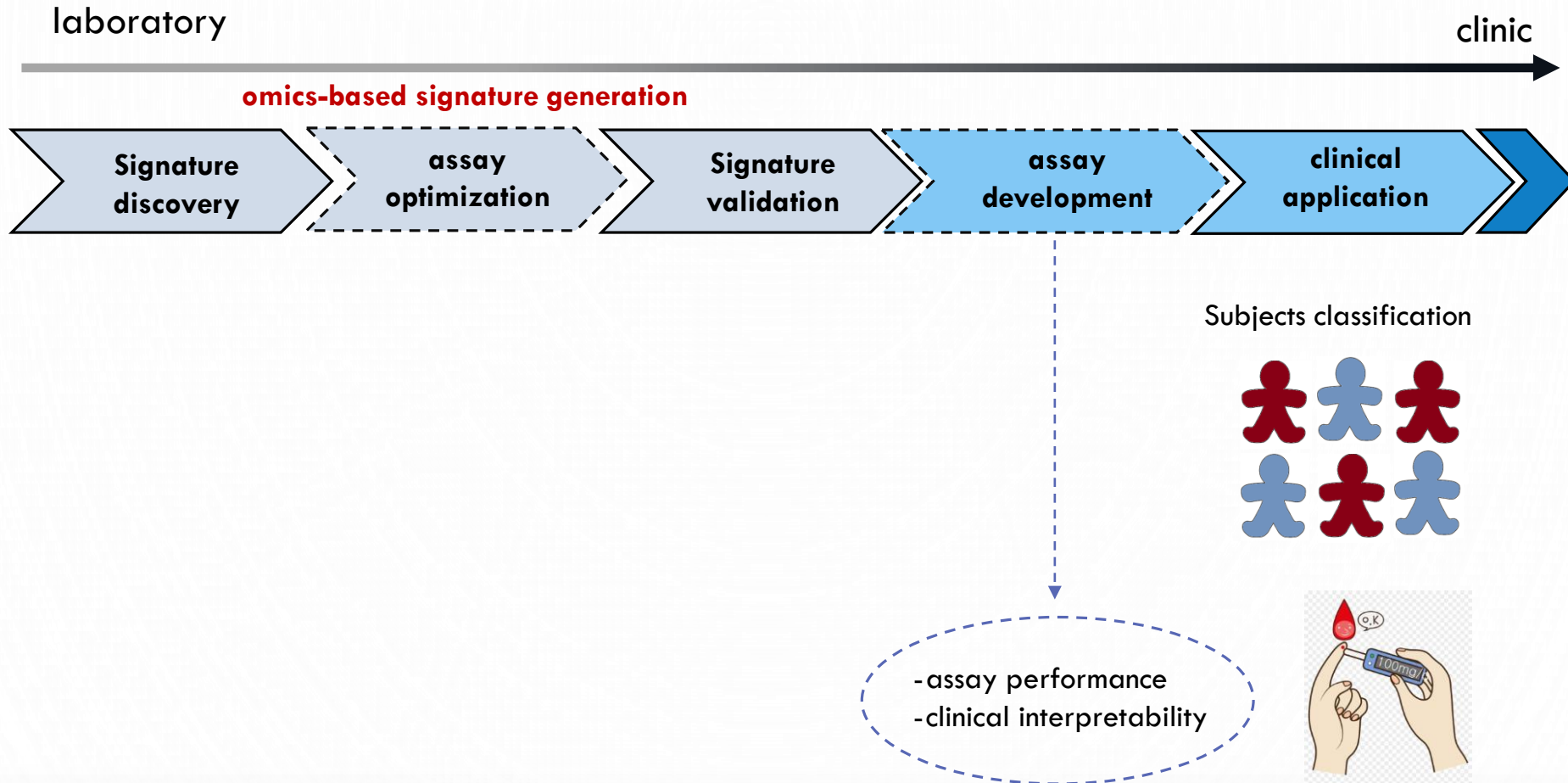
Serena Di Cosimo, Valentina Appierto, Sara Pizzamiglio, Paola Tiberio, Marilena V Iorio, Florentine Hilbers, Evandro de Azambuja, Lorena de la Pena, Miguel Ángel Izquierdo, Jens Huober, José Baselga, Martine Piccart, Filippo G. De Braud, Giovanni Apolone, Paolo Verderio, and Maria G Daidone

DOI: 10.1158/1078-0432.CCR-18-2507 [Check for updates](#)

# From biomarker discovery ... to biomarker validation

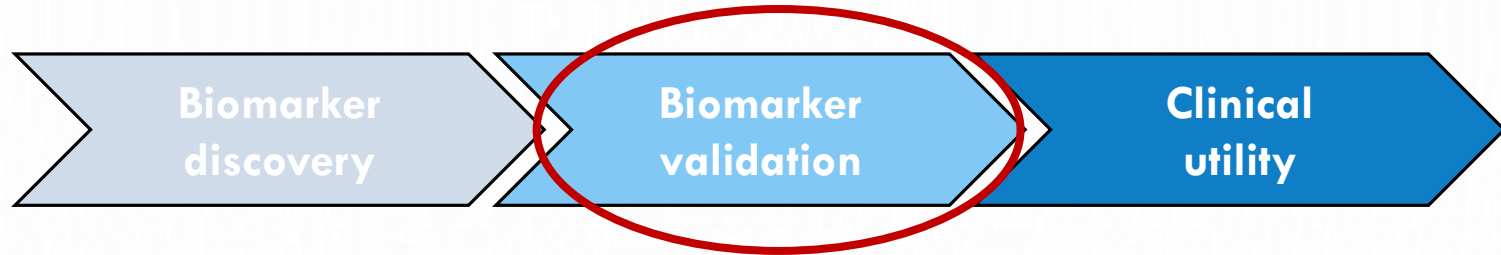


# Biomarker validation (1)





# Biomarker validation (2)



- ✓ **Analytical validation**: demonstrates the **robustness** (accuracy, precision, reproducibility) of the test

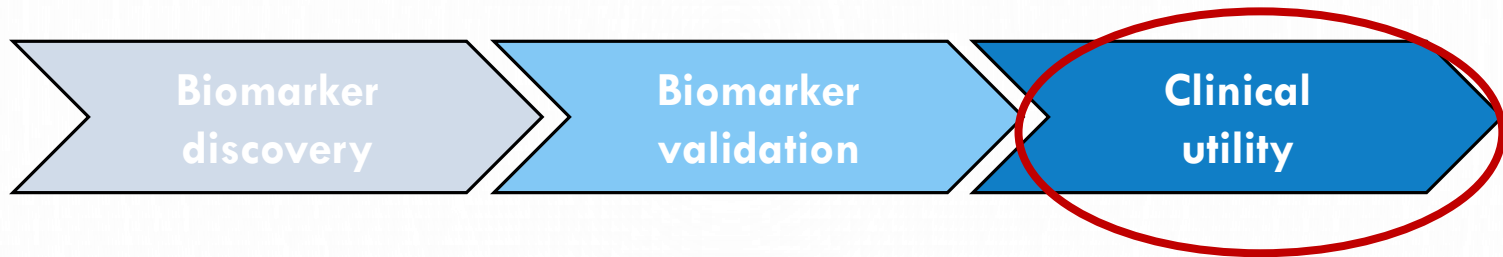
*how well does the test measure what it claims to measure?*

- ✓ **Clinical Validation**: demonstrates the **effectiveness** of the test

*how relevant is the test measurement to the clinical condition?*

*is the biomarker related to the clinical endpoint?*

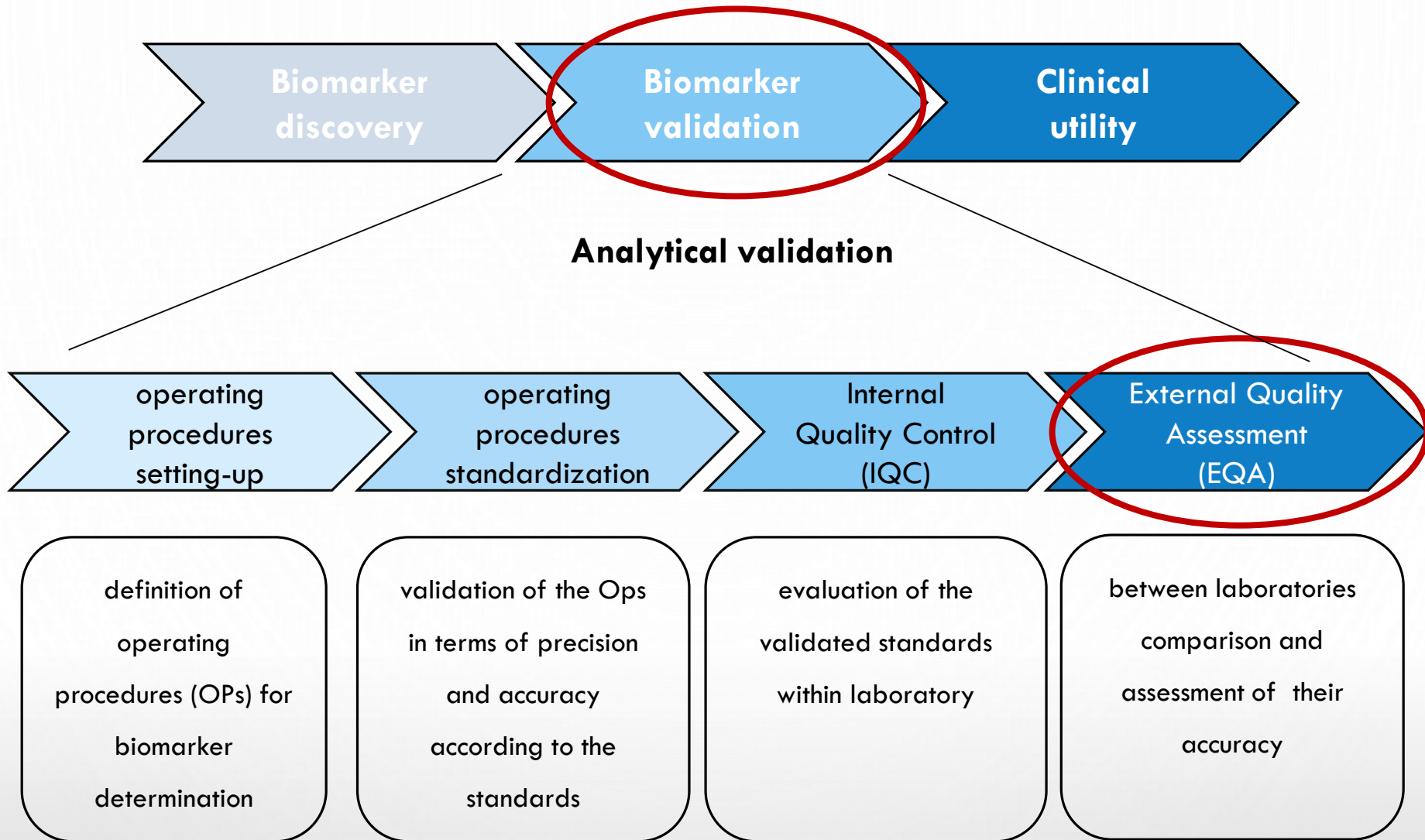
# Clinical utility



The conclusion that a given use of a medical product **will lead to a net improvement in health outcome** or provide useful information about diagnosis, treatment, management, or prevention of a disease.

*Does use of the biomarker result in patient benefit?*

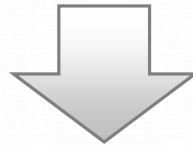
# Focus on the analytical validation



# External Quality Assessment (EQA)

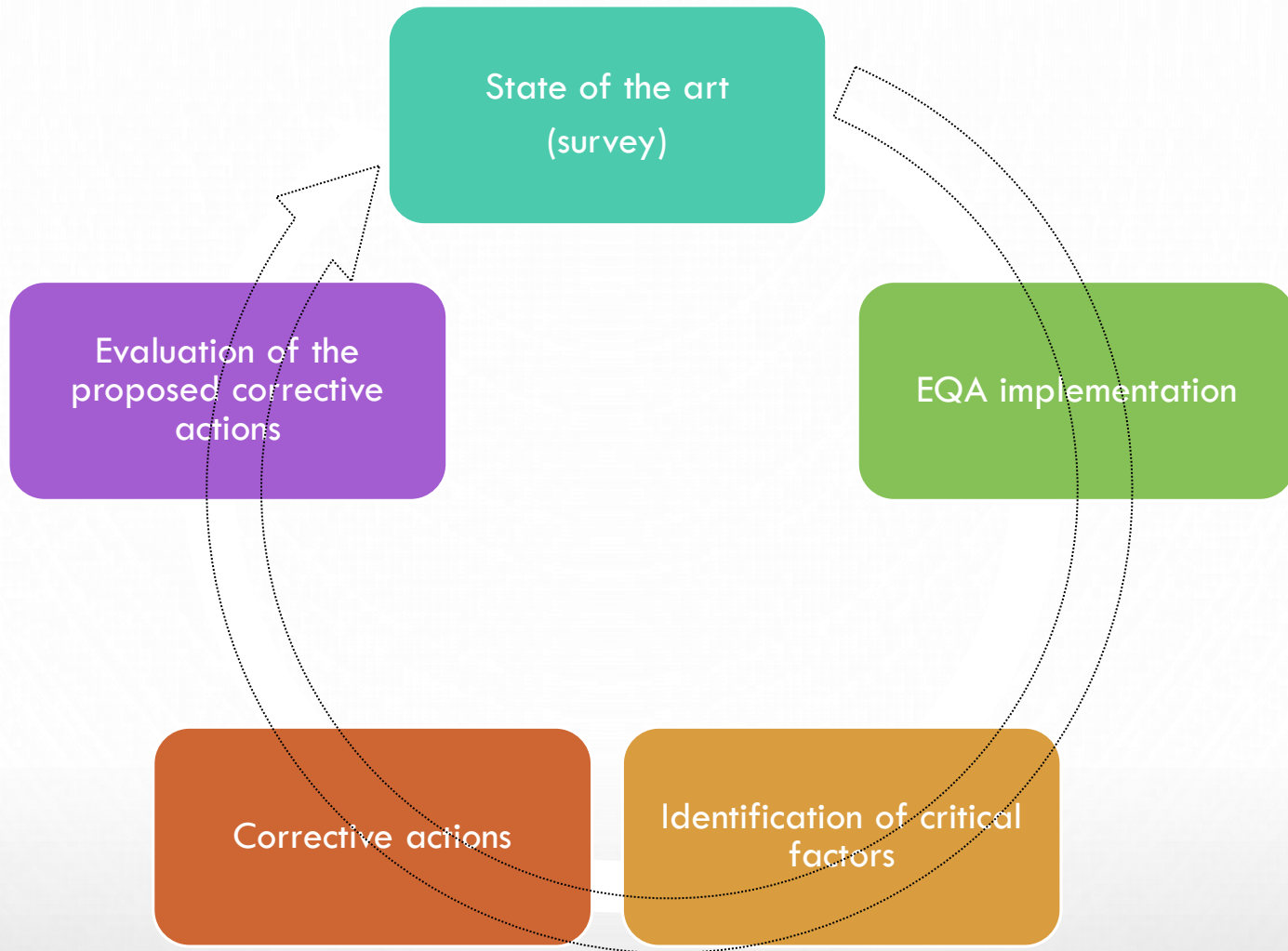
## Purpose of EQAs:

- (a) evaluation of laboratory performance for specific tests and its continuous monitoring
- (b) identification of inter-laboratory differences
- (c) evaluation of method/diagnostic system performances
- (d) degree of comparability between methods/diagnostic systems
- (e) monitoring of the success of harmonization/standardization efforts for improving results comparability

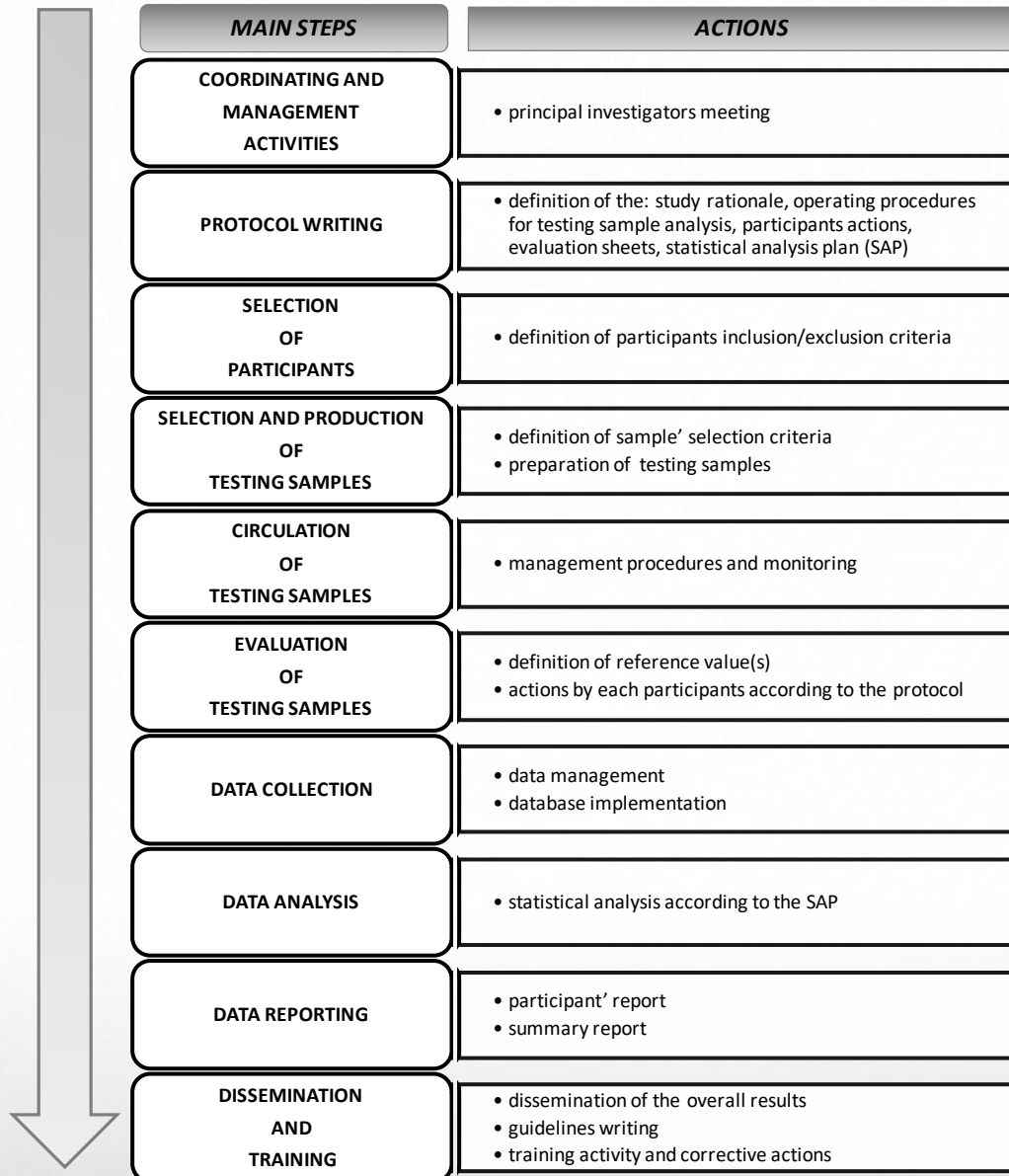


- ✓ can be organized in various biomedical research field
- ✓ can be focused on the **pre-analytical, analytical or post-analytical** phase of biomarker determination

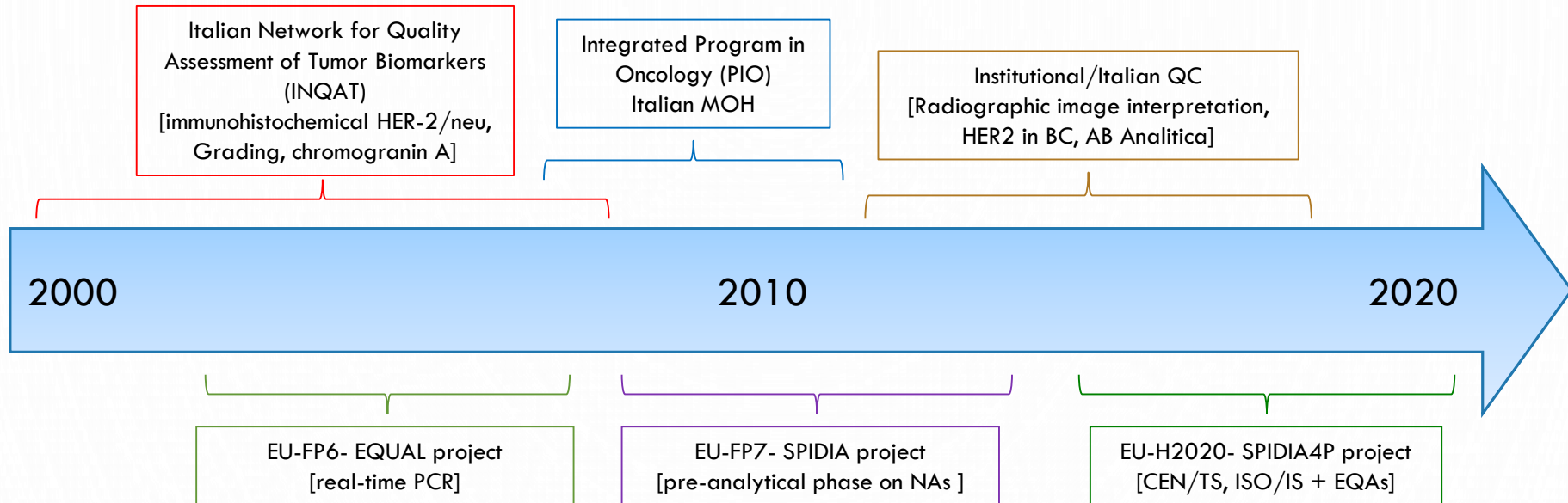
# Dynamicity of the EQA schemes



# EQA: scheme design



# Our experience in EQAs



**“Preanalytical errors still account for nearly 60%-70% of all problems occurring in laboratory diagnostics, most of them attributable to mishandling procedures during collection, handling, preparing or storing the specimens”.**

[Lippi G. et al. Clin Chem Lab Med. 2011; 49:1113-26]

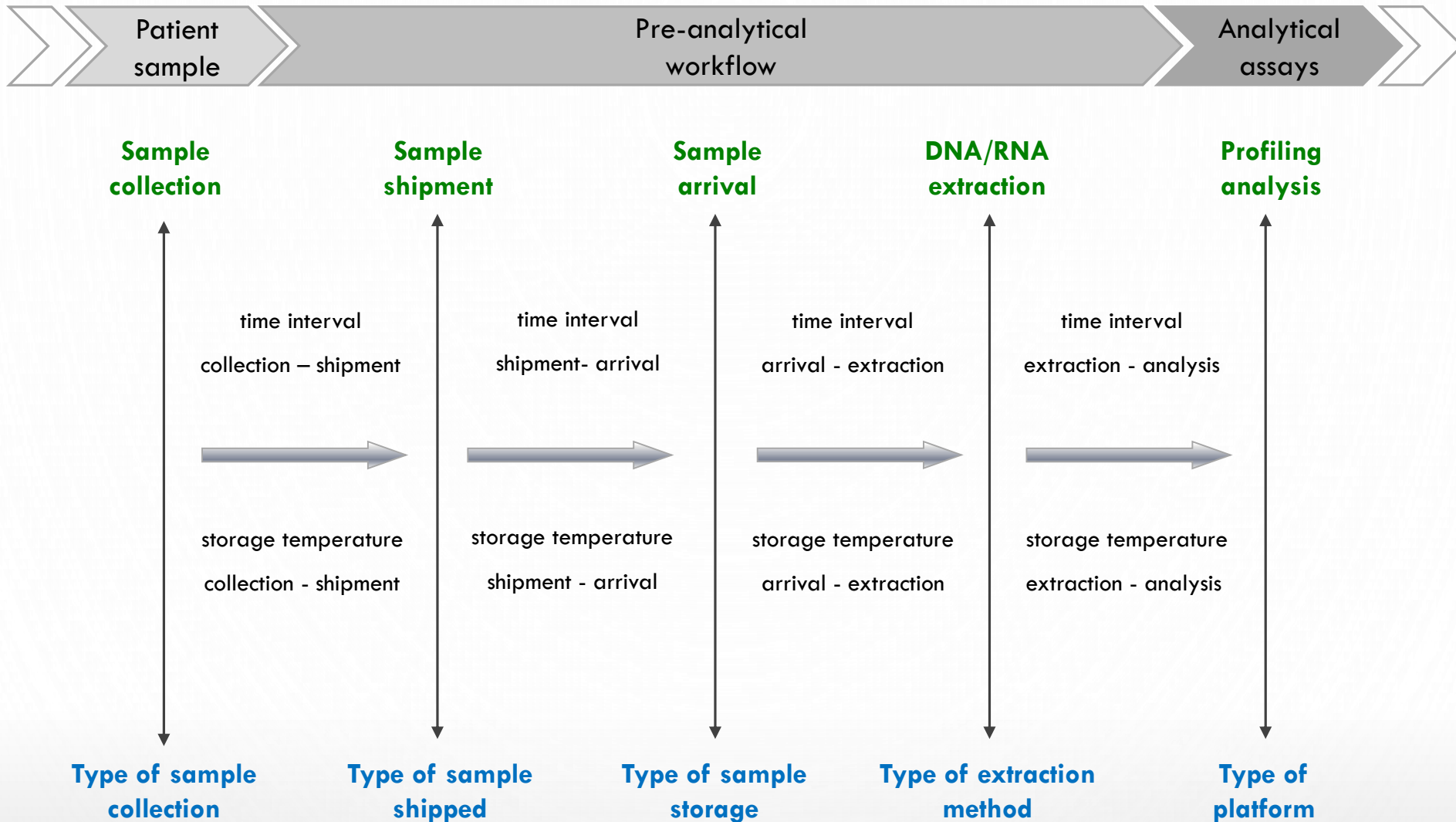
NAs: Nucleic Acids;

CEN/TS: Comité Européen de Normalisation/ Technical Specification

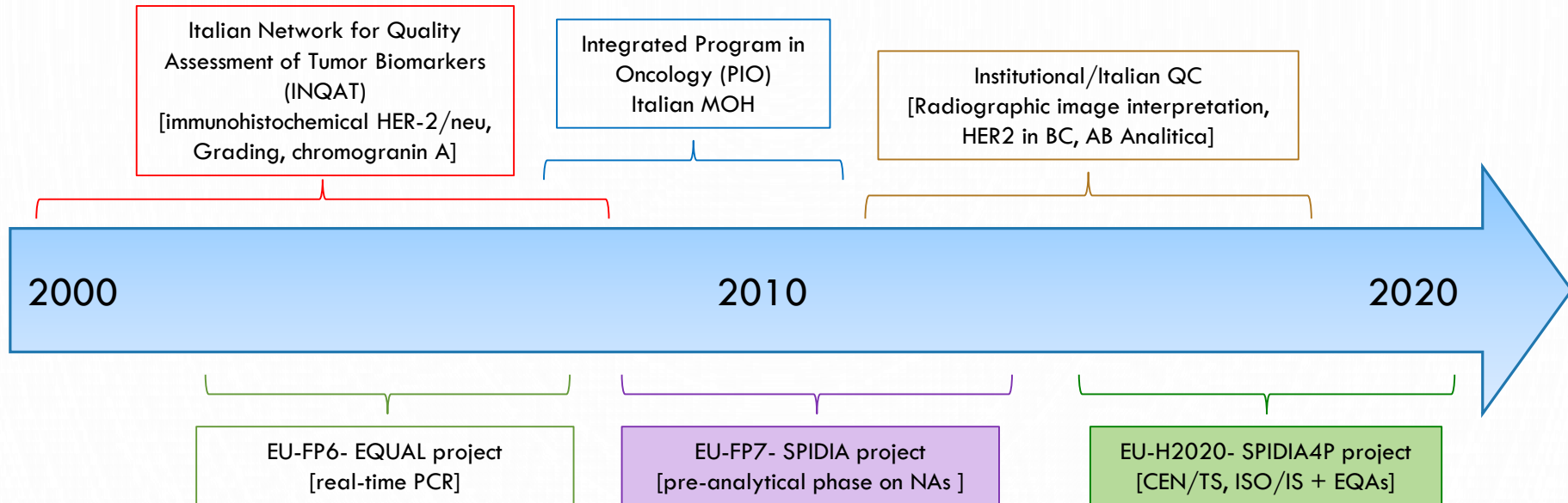
ISO/IS: International Organization for Standardization/ International Standards



# Pre-analytical workflow



# Our experience in EQAs



**“Preanalytical errors still account for nearly 60%-70% of all problems occurring in laboratory diagnostics, most of them attributable to mishandling procedures during collection, handling, preparing or storing the specimens”.**

[Lippi G. et al. Clin Chem Lab Med. 2011; 49:1113-26]

# The EU SPIDIA and SPIDIA4P projects



**SPIDIA** Standardisation and Improvement of generic pre-analytical tools and procedures for in-vitro diagnostics

**SPIDIA4P**

Home About Us About the Projects News and Press Events and Trainings Publications Links

**NEWSLETTER**  
Subscribe to our newsletter to receive latest news about the project

**CONTACT US**  
Visit our contact form to contact the consortium and to submit comments and questions about this website.

**ABOUT SPIDIA AND SPIDIA4P**

SPIDIA was a 4.5-year project funded by the European Union FP7 programme. It brought together 16 leading academic institutions, international organisations and life sciences companies, coordinated by QIAGEN GmbH. The project tackled the standardisation and improvement of pre-analytical procedures for in-vitro diagnostics. Various new pre-analytical technologies were developed. Within the CEN/Technical Committee 140 for "in vitro medical devices", SPIDIA's results enabled to develop and introduce the first 9 CEN Technical Specifications (CEN/TS) for pre-analytical workflows in Europe.

The SPIDIA4P project builds on SPIDIA's results and is funded by the European Union's Horizon 2020 research and innovation programme. The consortium of 19 highly experienced partners from private industry including SMEs, public institutions and one European Standards Organisation is again coordinated by QIAGEN GmbH. It plans to initiate, develop and implement a comprehensive portfolio of an additional 14 pan-European pre-analytical CEN/TS and ISO/15 documents as well as external quality assessment schemes (EQAs), addressing the important pre-analytical workflows applied to personalised medicine.

+++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++

On September 13, 2017, during the Global Biobank Week in Stockholm, Sweden, Dr. Uwe Oelmüller, QIAGEN, project-coordinator of SPIDIA and SPIDIA4P, was awarded for his comprehensive merits on pre-analytical standardisation in the EU.

\*\*\*\*\*

SPIDIA paper on PAXgene Tissue among the top 10% most cited PLOS ONE articles:  
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027704>

\*\*\*\*\*

Here you can find the list of the 9 introduced CEN Technical Specifications (CEN/TS) for pre-analytical workflows, developed on the basis of SPIDIA results.

For detailed background information and training on the CEN/TS, as well as information on what to consider for your workflow to be compliant, please check the 1 day training course announcement of SPIDIA/SPIDIA4P partner TATAA Biocenter: "New ISO and CEN Technical Specifications for the Pre-analytical Process in Molecular Diagnostics".

\*\*\*\*\*

SPIDIA4P is presented within the latest issue (#6) of the BBMRI-ERIC Biobanks Europe Magazine.

+++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++ LATEST NEWS +++

\*\*\*\*\*

The SPIDIA project has received funding under the Seventh Research Framework Programme of the European Union, FP7-HEALTH-2007-1.2.5, under grant agreement no. 22205. The SPIDIA4P project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 73312.

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[www.spidia.eu](http://www.spidia.eu)

- **SPIDIA** (Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics) was a 4.5-year project funded by the EU-FP7 programme (GA n. 222916, 2008-2013)

- **SPIDIA4P** (Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics for personalize medicine) is a 4-year project funded by the H2020 programme (GA n. 73312, 2017-2020)

- 19 partners coordinating by QIAGEN
- built upon SPIDIA's results



SPIDIA4P Kick-off Meeting, 30<sup>th</sup> Jan – 1<sup>st</sup> Feb 2017, Hilden

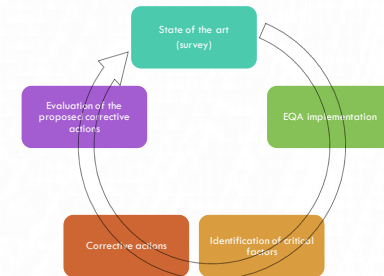
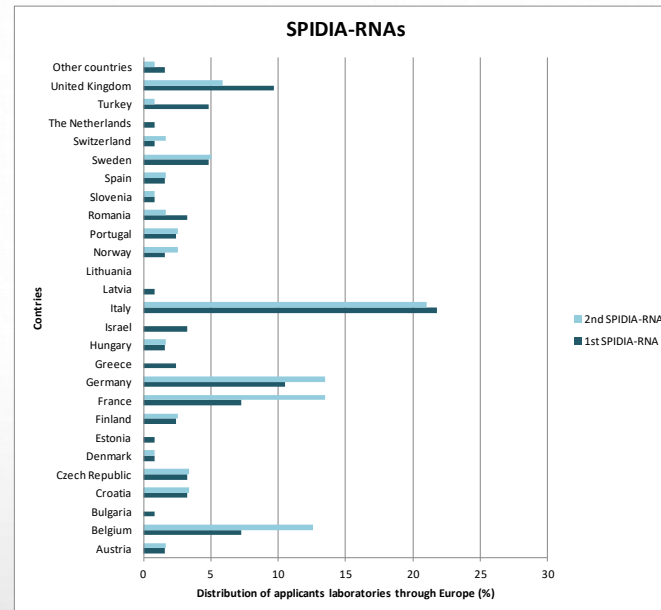
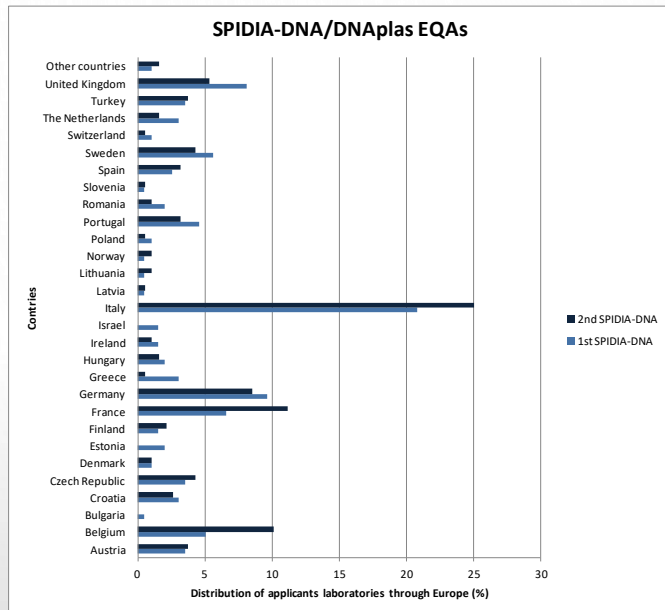
# SPIDIA blood EQAs: participant laboratories

2 runs of EQAs for blood-DNA/DNA-plas and blood-RNA

	SPIDIA – DNA/DNAplas		SPIDIA - RNA	
	applicants	effective	applicants	effective
1st EQA	197 <sup>(a)</sup>	183	124	93
2nd EQA	188 <sup>(b)</sup>	174	122	109

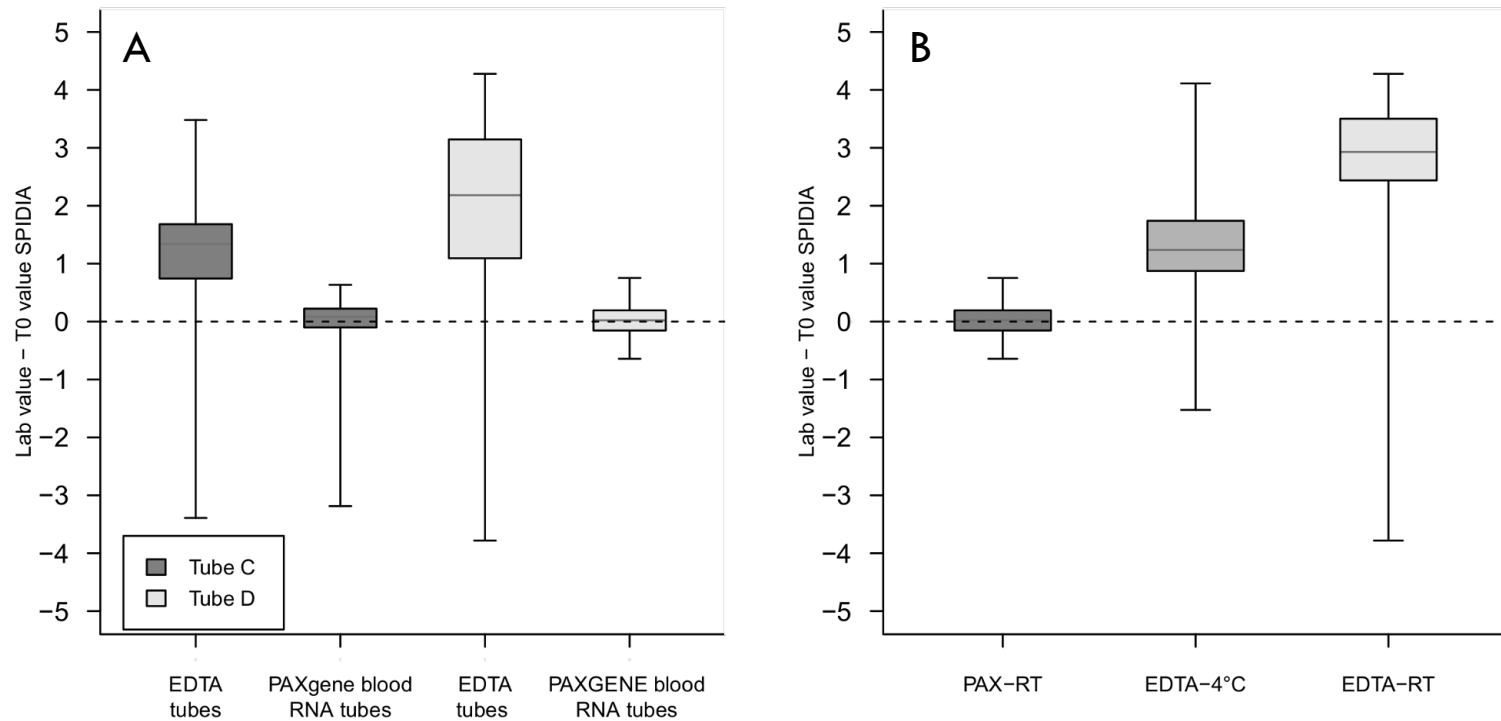
(a) 130 labs took part to the SPIDIA-DNA, only; 67 labs to both SPIDIA-DNA and SPIDIA-DNAplas EQAs

(b) 127 labs took part to the SPIDIA-DNA, only; 61 labs to both SPIDIA-DNA and SPIDIA-DNAplas EQAs



# SPIDIA blood EQAs: some results

Participants were asked to extract RNA from Tube C (RNA C) immediately upon arrival of the tubes and from Tube D (RNA D) 24 h after Tube C. PAXgene Tube D was stored at RT while EDTA Tube D was stored either at RT or at 4°C according to a randomized scheme.



Overall distribution of IL8 according to blood collection tube (panel A) and to storage temperature/collection tube (panel B). The box horizontal sides identify the 25th and 75th centile, the horizontal line inside the box the median, the two whiskers correspond to minimum and maximum, and the dashed line indicates the T0 value zero.

# SPIDIA: the achieved goals

Development of **9 CEN Technical Specifications (CEN/TS) for pre-analytical workflows** in Europe, within the CEN/Technical Committee 140 for “In vitro medical devices”:

- ✓ CEN/TS 16826-1, snap frozen tissue – Part 1: Isolated RNA
- ✓ CEN/TS 16826-2, snap frozen tissue – Part 2: Isolated proteins
- ✓ CEN/TS 16827-1, FFPE tissue – Part 1: Isolated RNA
- ✓ CEN/TS 16827-2, FFPE tissue – Part 2: Isolated proteins
- ✓ CEN/TS 16827-3, FFPE tissue – Part 3: Isolated DNA
- ✓ CEN/TS 16835-1, venous whole blood – Part 1: Isolated cellular RNA
- ✓ CEN/TS 16835-2, venous whole blood – Part 2: Isolated genomic DNA
- ✓ CEN/TS 16835-3, venous whole blood – Part 3: Isolated circulated cell free DNA from plasma
- ✓ CEN/TS 16945, metabolomics in urine, serum and plasma





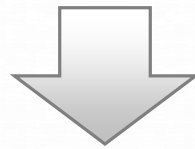


### **III. Concluding remarks**



It is important in the –omics era to have:

- reliable biomarkers easily translatable in the clinic
- guidelines for the whole biomarker determination process from sample collection to biomarker detection and analysis



SPIDIA4P aim to implement comprehensive portfolio of 21 pre-analytical CEN/TS to be developed into global ISO/IS documents

**THANK YOU!**