Biomarkers: Biology vs Bioanalysis

Tim Sangster, Executive Director

Celerion Inc.

8th Nov 2021

Outline

- Celerion – Introduction
- Biomarkers – and drug development
- Biology – challenges
- Bioanalysis – opportunities and challenges

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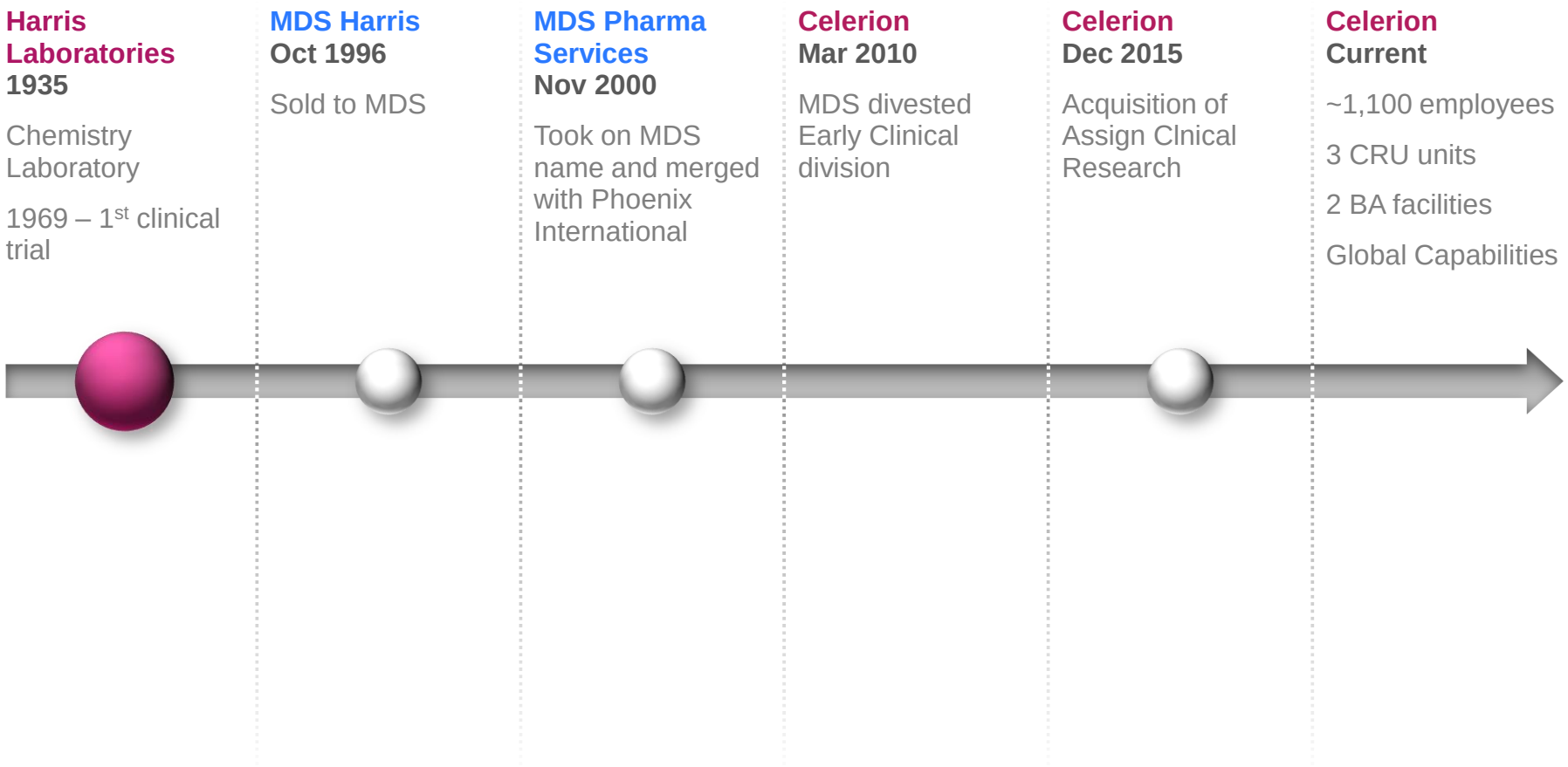
Celerion - History



Global Bioanalytical Services

Lincoln, Nebraska, USA

- 30,000 sq. ft.
- > 40 years of operation
- Last FDA Inspection: January 2019 – ZERO observations

- LC-MS/MS (25 Systems) & ICP/MS
- Ligand Binding Services - Flow Cytometry, EliSpot, ELISA, MDS, SIMOA
- Immunogenicity testing
- Clinical biomarkers
- Genomic (qRT-PCR)
- Genetic (PCR) Assay Services

Zurich, Switzerland

- 40,000 sq. ft. (4,300 sq. m.)
- > 35 years of operation
- SwissMedic certified (OECD)
- Last FDA Inspection: Feb 2019 – ZERO observations

- LC-MS/MS (13 systems)
- Metabolite Identification by HRMS
- Ligand Binding Services (ELISA, ECLIA, RIA, SIMOA)
- Immunogenicity testing and Alpha Lisa (ADA, nAb)

Information Management Drives the Speed of Science

Data Capturing and Data Integrity

Thermo Fisher Watson™ 7.6 LIMS

- LIMS configured on the Citrix Metaframe™ Server
- Bi-directional interface with analytical instruments
- Tracking of samples, use of barcoded labels
- Export of data for reporting

Vaisala viewLinc™ 4.3.5

- CMS system of all temperature controlled areas
- Monitoring of temperature and humidity
- Alert and reporting function

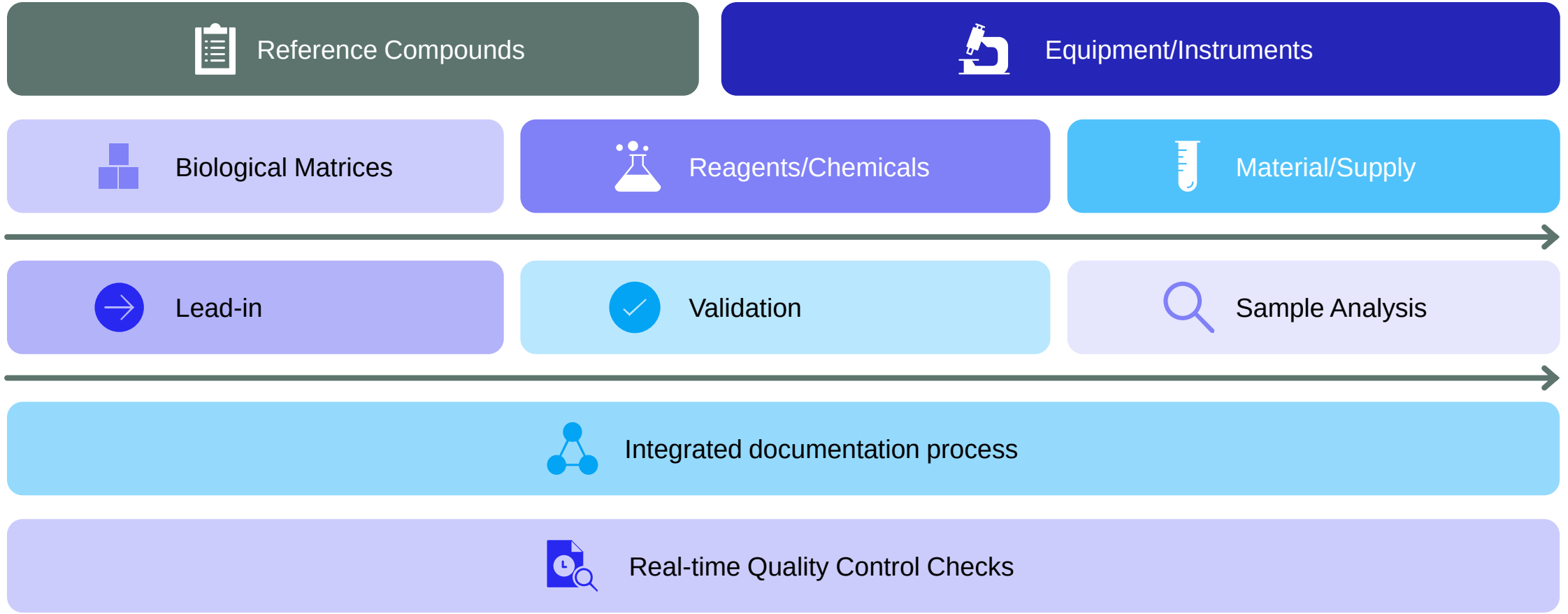
Terrington Data Management Labnotes™ 7.16 (ELN) Release 11

- One of the first global CROs to have an ELN system
- All equipment, reagents and supplies are bar-coded
- Real-time QC
- Virtually paperless laboratory

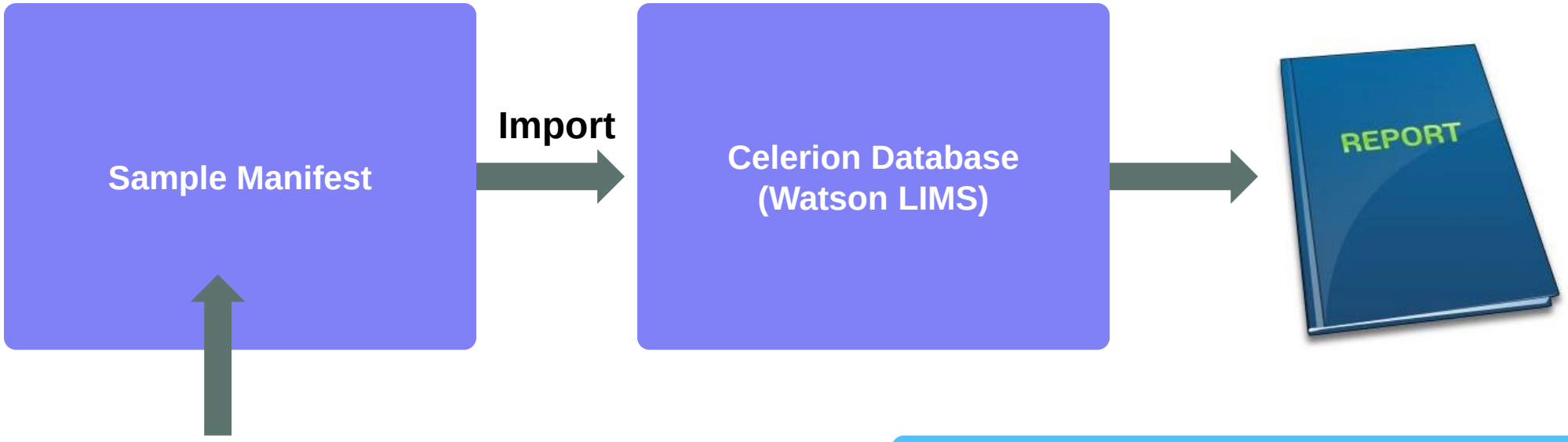
All computer/ automated systems are 21 CFR Part 11 compliant

Electronic Laboratory Notebook

Improved Process - Overall Integration



Data Traceability back to the original tube….



Barcoded Label Scan
(Celerion & Third Party)

Full Traceability from BarCode via

- Subject Number
- Visit No. / Timepoint
- Accession No.
- Result / Concentration

What Are Biomarkers?

- For the purposes of the this talk, a Biomarker is an endogenous substance, which can be measured, and for which it’s concentration correlates to the progression of a disease state or to a treatment regime.

However…

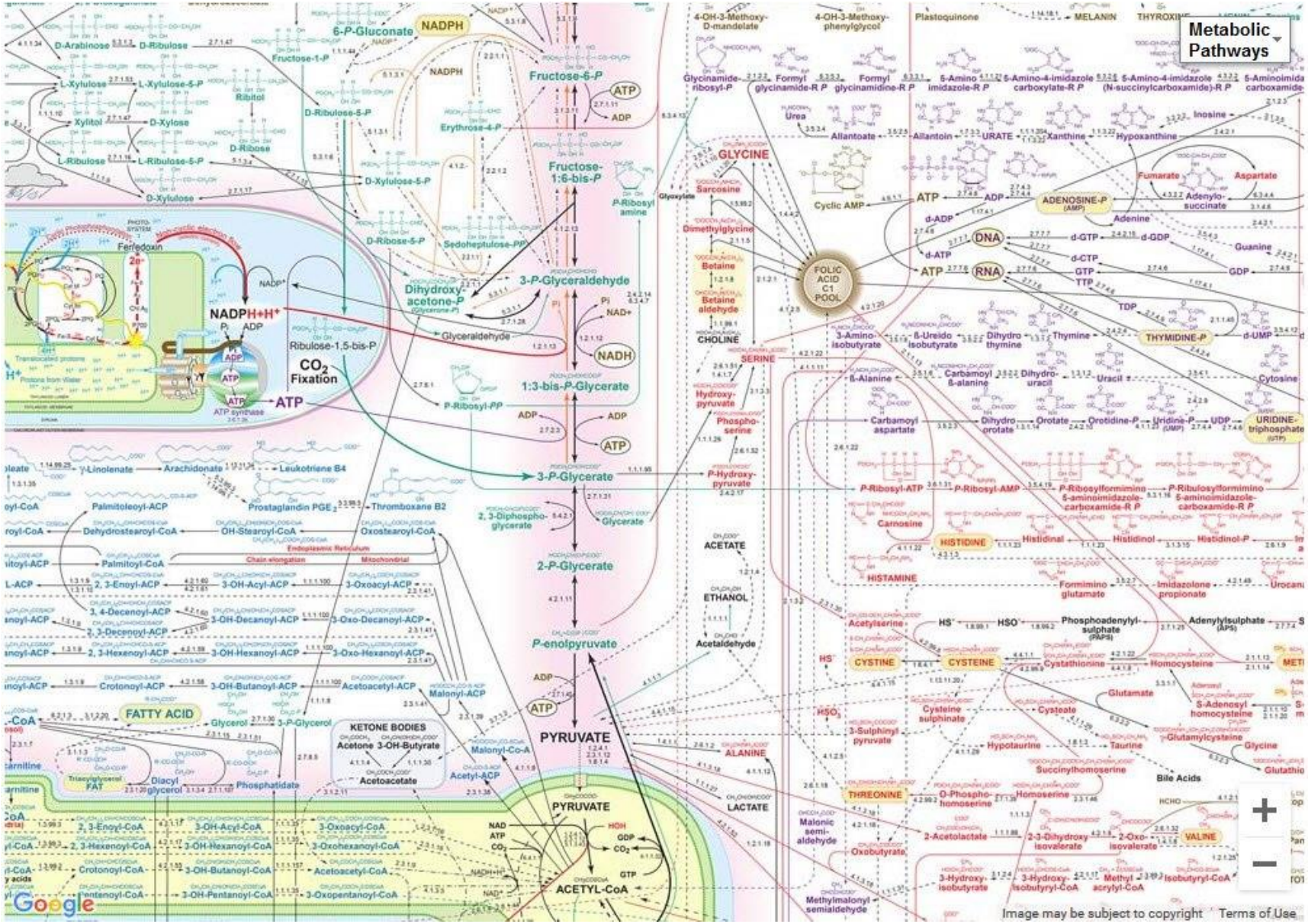
- However a more practical definition could be:

  “A Biomarker could be a substance, that may or may not be measurable, which may or may not change is response to a disease, and can depend on age, gender, time of day, and astrological sign…”

A Broad Category

- The Biomarker category is a broad one and covers 1000s of years of medical history, allegedly starting with sweet tasting urine (diabetes) and continuing today with regular advances in disease detection and monitoring as well as evaluating treatment across nearly all fields of medicine.

Biology



https://www.sigmaaldrich.com/GB/en/technical-documents/technical-article/research-and-disease-areas/metabolism-research/interactive-metabolic-pathways-map

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Biomarkers in Drug Development

- “This guidance helps sponsors of investigational new drug applications (INDs) or applicants of new drug applications (NDAs), abbreviated new drug applications (ANDAs), biologic license applications (BLAs), and supplements validate bioanalytical methods used in human clinical pharmacology, bioavailability (BA), and bioequivalence (BE) studies that require pharmacokinetic, toxicokinetic, or biomarker concentration evaluation. This guidance can also inform the development of bioanalytical methods used for nonclinical studies that require toxicokinetic or biomarker concentration data.’

- *Bioanalytical Method Validation: Guidance for Industry, FDA CDER CVM May 2018*

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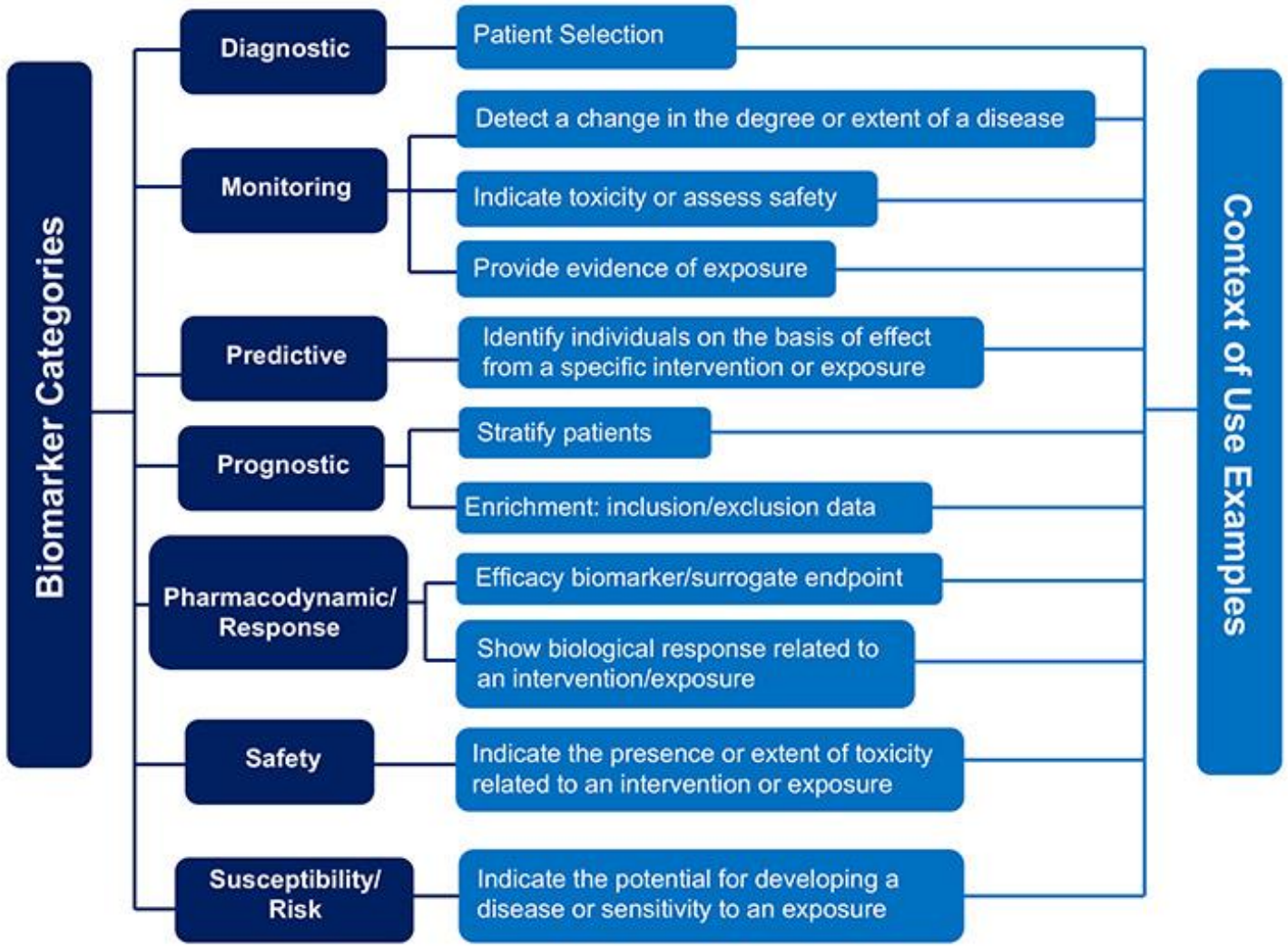
BEST Biomarker Category and Examples of Corresponding Drug Development Uses



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Examples of Biomarker Intended Use in Drug Development

- Defining inclusion/exclusion criteria
- Defining treatment allocation arms
- Cessation of a patient's participation in a clinical trial
- Establishing a drug's proof of concept in a patient population
- Supporting clinical dose selection
- Serving to enrich clinical trial for an event or population of interest
- Evaluating treatment response

- FDA.gov/drugs/biomarker-qualification-program/context-use

Common Examples Of Biomarkers

- Cholesterol – Coronary and Vascular Disease
- Insulin – Diabetes
- BRCA1 – Breast Cancer
- PSA – Prostate Cancer
- Rheumatoid factors – Rheumatoid Arthritis
- Bilirubin – Liver Function
- White blood cells – Infection

Biologic Qualification vs Analytical Validation

- Biologic Qualification
  - Demonstrating the marker tracks the biologic change that it is intended to and inform on the impact of a therapy of disease state

BIOLOGY

- Analytical Validation
  - Demonstrating the assay measure the analyte of interest and what are the limitations of the measurement.

BIOANALYSIS

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Biology – Clinical Laboratories

ASSAY	REF RANGE	UNITS	DISEASE STATE
Albumin	**4.0 - 5.1**	g/dL	Hepatic
Bicarbonate, Urine	**TBD**		Renal
Bilirubin, Total	**0.2 - 1.6**	mg/dL	Hepatic
Calcium Urine	**0.0 - 300.0**	mg/dL	Renal
Chloride Urine	**110 - 250**	mEq/L	Renal
Cholesterol Total	**119 - 268**	mg/dL	Lipidemia
Creatinine Serum	**0.69 - 1.20 (M)** **0.50 - 0.90 (F)**	mg/dL	Renal
Creatinine Urine	**14 - 326**	mg/dL	Renal
Creatinine Serum, Enzymatic	**0.69 - 1.20 (M)** **0.50 - 0.90 (F)**	mg/dL	Renal
Creatinine Urine, Enzymatic	**14 - 326**	mg/dL	Renal
Cystatin C	**0.5 - 1.1**	mg/dL	Renal
Ethanol	**0.0 - 10.0**	mg/dL	Hepatic
Free Fatty Acids	**0.00 - 0.89**	mEq/L	Lipidemia/Diabetic
HDL Cholesterol	**30 - 93**	mg/dL	Lipidemia
Inorganic Phosphorus Urine	**0.0 - 500.0**	mg/dL	Renal
LDL Cholesterol	**58 - 188**	mg/dL	Lipidemia
Lipase	**14 - 91**	U/L	Pancreatic
Magnesium, Urine	**0.6 - 13.7**	mg/dL	Renal
Potassium Urine	**25 - 120**	mEq/L	Renal
Sodium Urine	**40 - 220**	mEq/L	Renal
Triglycerides	**40 - 287**	mg/dL	Lipidemia
Total T3	**84 - 160**	ng/dL	Endocrine
Total T4	**4.6 - 11.0**	ug/dL	Endocrine
TSH	**0.4 - 4.0**	uIU/mL	Endocrine

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Regulatory Language

- When it comes to analyzing Biomarkers there are some terms we should define upfront because they are key to developing effective methods:

- “**Context of use**”

- “**Fit for purpose**”

Context Of Use

- The Context of Use (CoU) for a Biomarker is defined by the FDA as "***the circumstances under which the drug development tool is to be used in drug development and regulatory review***"

- In other words, what is the purpose of measuring this biomarker?

- The CoU is critically important to analysis and dictates the level of rigor required when developing and validating a method.

- The CoU may also evolve over time as more experiment data is collected, and as such Biomarker methods should likewise be expected to evolve.

Context Of Use In Validations

- When the CoU is only in early phase exploratory research work it may only be necessary to detect the presence or absence of a biomarker above a set threshold. Very simple methods.

- However, for a Biomarker assay that is expected to replace an existing safety assessment a fully validated method that reliably, accurately and precisely measures concentrations across a broad range would be required.

- In all cases, the analytical method should be **Fit For Purpose**, depending on the **Context of Use**.

FFP – Fit For Purpose

Paraphrasing from the FDA BMV May 2018:

- The fit-for-purpose (FFP) concept states that the level of validation should be appropriate for the intended purpose of the study.
- For assays intended to support early drug development (e.g., candidate selection, go-no-go decisions, proof-of-concept), the sponsor should incorporate the extent of method validation they deem appropriate.
- Pivotal studies that require regulatory decision making for approval, safety or labeling, such as BE or pharmacokinetic studies, should include bioanalytical methods that are fully validated.

Focus On Analysis

- For Biomarkers that are well categorized, such as those that have several decades of experimental history, like insulin, analysis is relatively straight forward, you just grab an off the shelf commercial kit and follow the instructions.

- Validation of these methods is also fairly routine, however there are still areas that require special attention, such as stability and parallelism.

Using Biomarker Kits

«Biomarker Kits are only good for measuring kit components – and sometimes not even then»

– Harley Williams

- Kits need to be verified against known external samples
- Stability testing with spiked QCs only tells you how stable the kit reference item is, and says nothing about the actual stability of the samples.

Commercial Diagnostic Kits

- Diagnostic kits are generally developed for use as clinical diagnostic tools - their suitability for use in such studies should be demonstrated
- Diagnostic kit validation data provided by the manufacturer may not ensure that the kit method is reliable for drug development purposes.
- Site-specific validation should be performed
- Calibration curve with a sufficient number of standards across the calibration range
- Actual QC concentrations should be known

Commercial Diagnostic Kits

- Standards and QCs should be prepared in the same matrix as the subject samples
- If the analyte source (i.e. reference standard) in the kit differs from that of the subject samples (e.g. the sample is a protein isoform of the reference standard), testing should evaluate differences in assay performance of the kit reagents
- If multiple kit lots are used within a study, lot-to-lot variability and comparability should be addressed for any critical reagents
- Individual batches using multiple assay plates (e.g., 96-well ELISA plates) should include sufficient replicate QCs on each plate to monitor the accuracy of the assay.

Novel Biomarkers

Developing and validating methods for novel Biomarkers on the other hand can be quite another story:

- Experimental properties, such as stability, may be unknown
- Baseline levels may vary depending on population
- Commercial kits my not exist, or may not be reliable
- Additional development time may be required

Main Message From The EBF

"Biomarker assays are NOT PK assays and they don't want to become PK assays!"

"They should not be **developed** like PK assays

They should not be **validated** like PK assays"

"Comparing PK and Biomarker assays is like comparing Robots and Aliens".

- Marianne Scheel Fjording

Communication Is Key

- Before we start any Biomarker work we need to know exactly what is required for the study:

- **Expected concentration ranges**
  - *In Normal or Disease state, different Populations, Age, Gender, etc*
- **Expected CHANGES in concentration**
  - *Up or Down? Large or Small changes? Is there a safety limit?*
- **Expected duration**
  - *Is stability a factor? Will reagents remain available?*
- **What is the Purpose of testing this Biomarker?**
  - For Efficacy Testing or Safety Data? Or just Exploratory?

Understanding the Biomarker

- Physiological variability needs to be understood first. What is normal, what is not?
- Physiological variability needs to be > assay variability.
- If small changes are expected a very sensitive assay is needed
- If large changes are expected then accuracy may be completely irrelevant.

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Biomarker Ranges

- Are we only interested in Cmax values or changes from baseline?

- If accuracy is irrelevant then instead of reporting mass units (pg/mL) we could be reporting:
  - %Change from baseline
  - “Low Level” vs “High Level”
  - Titer values with LLOQ as cut off

Biomarker Validation

«Avoid over validation – don't aim for a PK assay!»

- Philip Timmerman

- Understand the Biomarker, understand the needs of the study and then evolve the method together.
- Biomarker methods take more time and shouldn't be rushed.
- Remember, comparing PK and Biomarker assays is like comparing Robots and Aliens.

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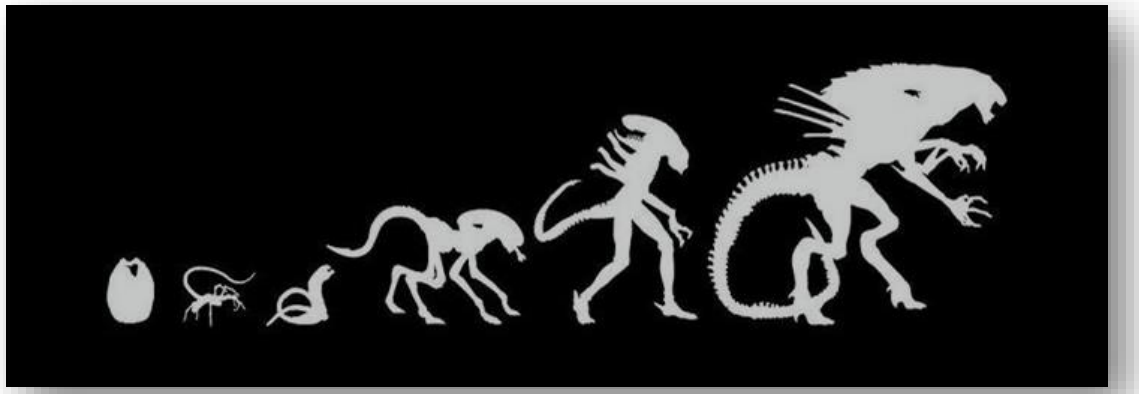
“Robots vs Aliens”

PK Assay Development

- Predictable
- Minimal changes

Biomarker Assay Development

- Evolves over time



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Approaches to Biomarker Method Validation

Do you have validated assay for Biomarker X?

Validated for what?

What are the relevant details we need to consider from the study to ensure we have the correct method?

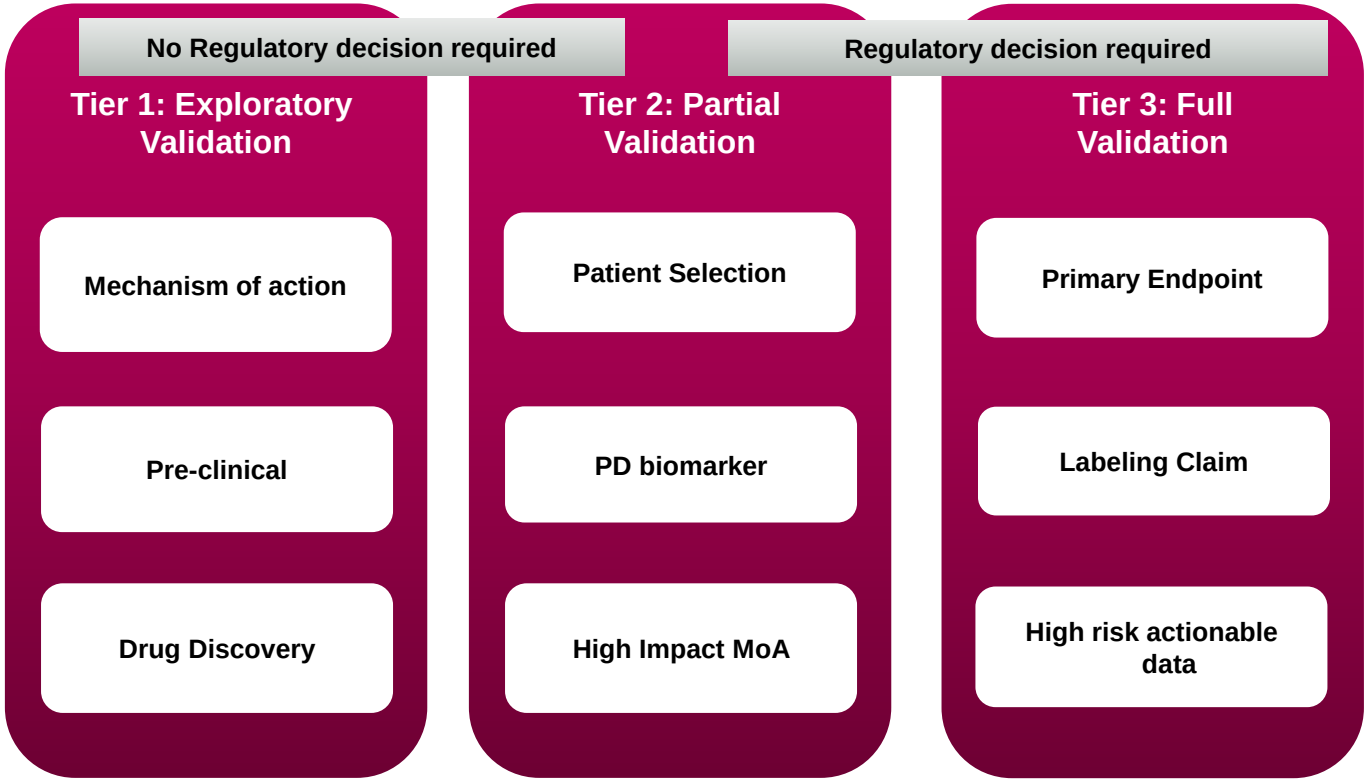
Biomarker Plan

- Context of Use - What is the intended use of the biomarker?
- Type of the data - quantitative vs. qualitative
- What is the threshold? Statistical plan?
- What is the suitable matrix?
- What is the underlying disease status?
- Endogenous interference
- Treatment interference
- Free vs. total
- Expected biological variance?
- Sensitivity requirement?
- Logistics - Sample volume, turn around time, throughput, etc.?
- Known stability/in-stability?

Analytical Validation

- Does the method measure the intended analyte? For example, does anything interfere with the measurement, and is the method specific or selective for the analyte?
- • What is the variability associated with these measurements? For example, what are the accuracy and precision of the method?
- • What is the range in measurements that provide reliable data? For example, what is the sensitivity of the method (e.g., what is the lower limit of quantitation (LLOQ) of the method, and what is the upper limit of quantitation the method (ULOQ)?)
- • How do sample collection, handling, and storage affect the reliability of the data from the bioanalytical method? For example, what steps need to be followed while collecting samples? Do the samples need to be frozen during shipping? What temperatures are required to store the samples, and how long can the samples be stored?

Tiered Biomarker Method Validation



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Three Tier Validation Approach

Parameter	Tier 1	Tier 2	Tier 3
Reference Material	Maintain and monitor quality of reference material. All new lots within a study are compared to the original lot of reference material	Maintain and monitor quality of reference material. All new lots within a study are compared to the original lot of reference material	Reference material must be compared to a well characterized standard (e.g., WHO, NIBSC, NIST), when available
Calibration Curve	≥6 calibrators using surrogate matrix fortified with reference material	≥6 calibrators using surrogate or study matrix fortified with reference materials	≥6 calibrators using surrogate or study matrix fortified with reference materials. Different COU may require different quantitation ranges
Parallelism	Recommended	Required	Required
Selectivity/Matrix Effect	Fortify 6 lots of matrix from normal subjects at 2 levels	Fortify 10 lots of matrix from normal subjects at 2 levels	Fortify ≥10 lots of matrix from normal and COU disease subjects each at a minimum of 2 levels

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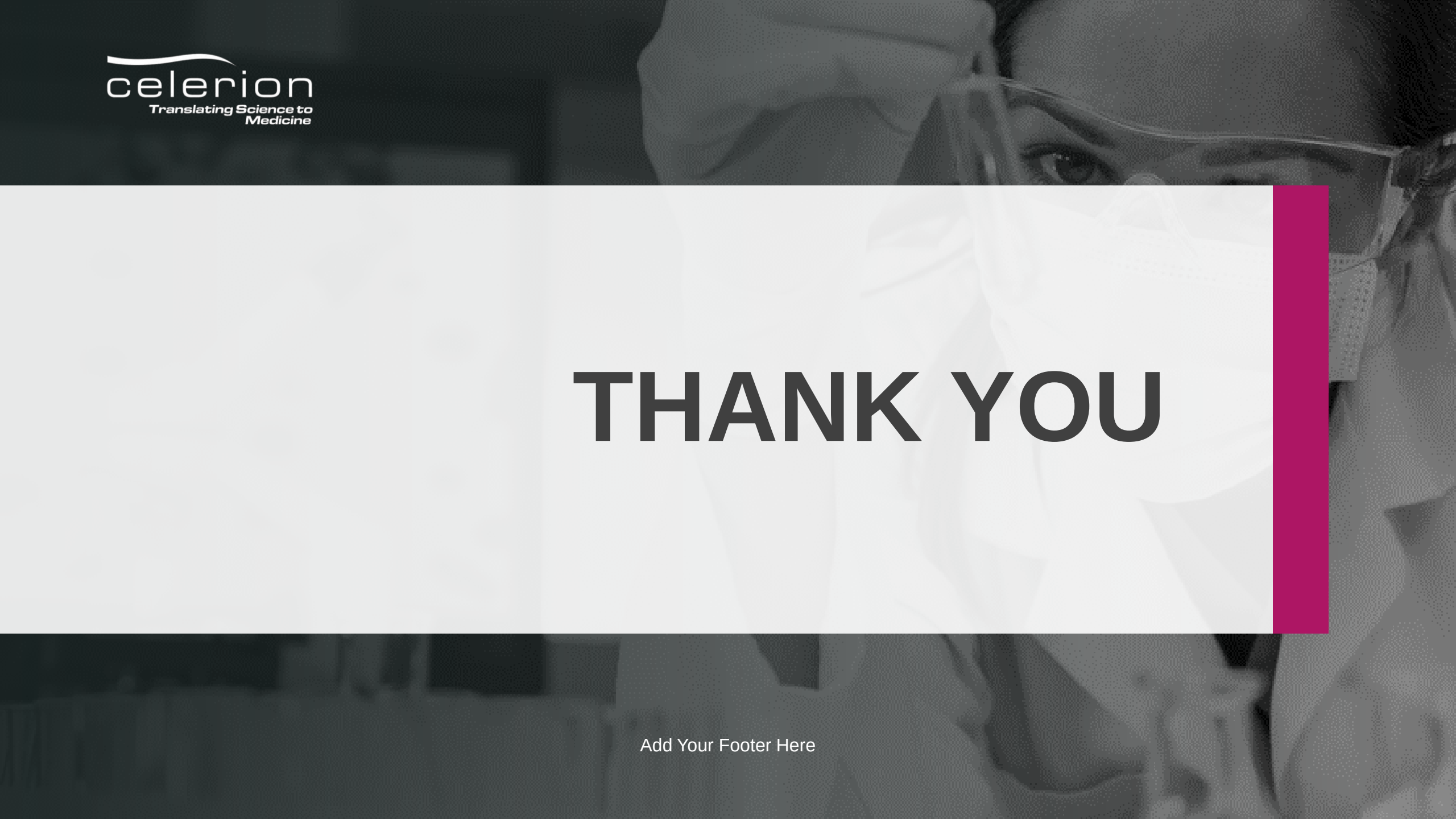
Three Tier Validation Approach

Parameter	Tier 1	Tier 2	Tier 3
Specificity/Cross-Reactivity for LBA	Recommended	Recommended	Required: compare structurally similar compounds
Precision and Accuracy	3 analytical runs	3 analytical runs	≥6 (3 for chromatography) analytical runs
QC Samples	3 surrogate matrix QC samples (e.g., LLOQ, low, mid, high, ULOQ)	≥3 surrogate matrix QC sample and/or endogenous QC samples from normal subjects. Endogenous QC samples from COU disease required only if difference in selectivity shown with normal subjects	Surrogate matrix QC samples and/or endogenous QC samples from normal subjects. Endogenous QC samples from COU disease required only if difference in selectivity shown with normal subjects
Dilution Linearity, Hook Effect for LBA	Recommended	Recommended	Required
Solution Stability	Scientific judgement	Scientific judgement	Long-Term Stability for Stock Solutions (LTSSS), Short-Term Stability for Stock Solutions (STSSS) of reference material
Matrix Stability	Scientific judgement	Freeze-Thaw and Short-Term Stability (FTSTS) on endogenous QC samples	Long-Term Stability (LTS), FTSTS on endogenous QC samples, SCHS
Lot-to-lot Variability of Critical Reagents	Recommended	Recommended	The validation protocol will specify critical reagents and requirements for testing

Summary

- Context of use is critical in determination of extent of validation
- When biomarker data will be used to support a regulatory decision, FDA expects a fully validated method as per BMV guidelines
- FDA approved diagnostic kit/methods may not be suitable for COU applied in drug development
- Collaboration between sponsor/principal clinical investigators, statistician and laboratory researchers is key to successful implementation of biomarker
- Engage Regulated BioAnalysis as early in the process as possible



THANK YOU

Case Study – Inflammatory Cytokines

- COU – Monitoring disease progress
- Validation tier 2
- Challenging assay

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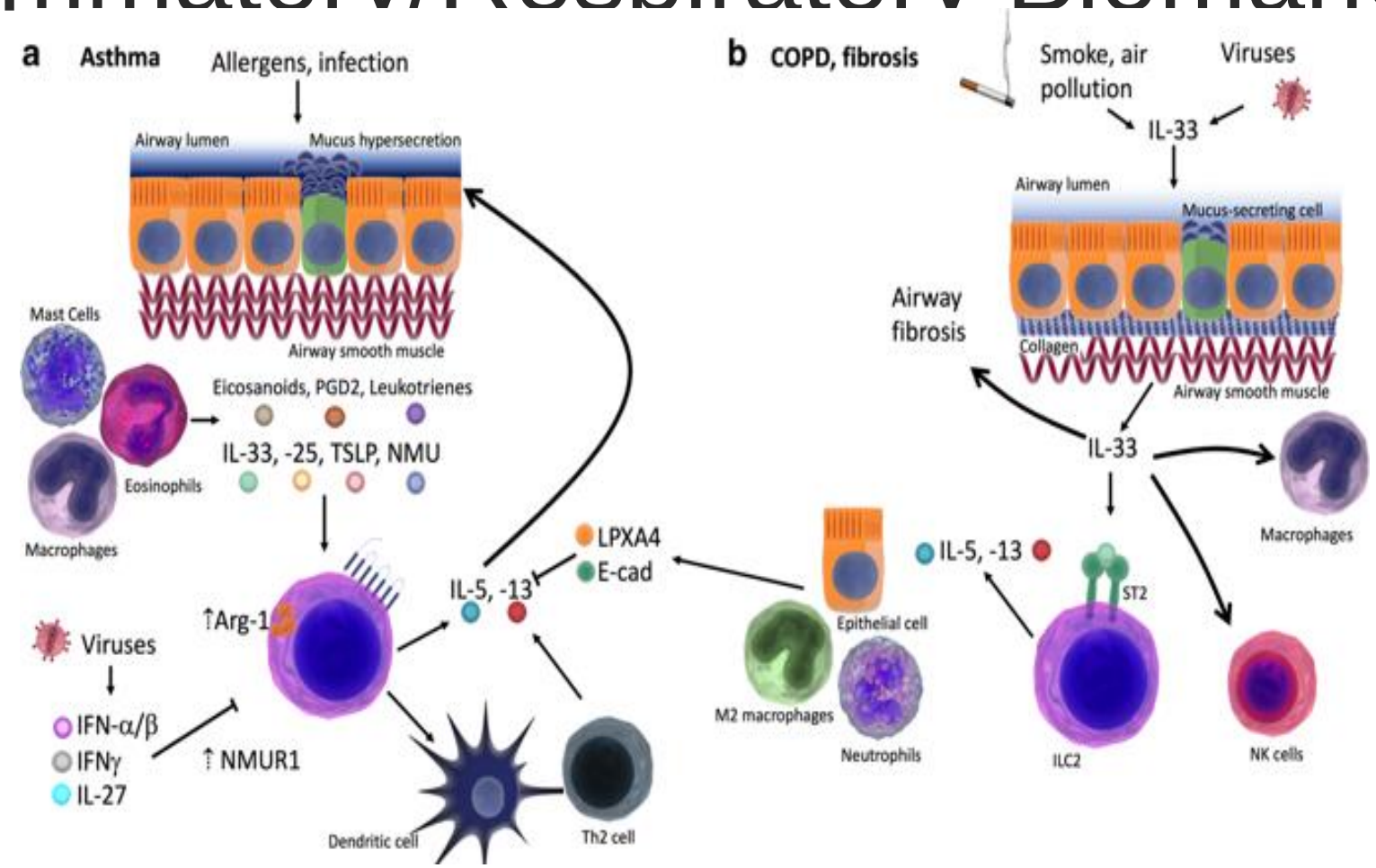
Inflammatory/Respiratory Biomarkers



Involved inflammatory Cytokines,IL-6, TNF-α, IL-13 and IL-5

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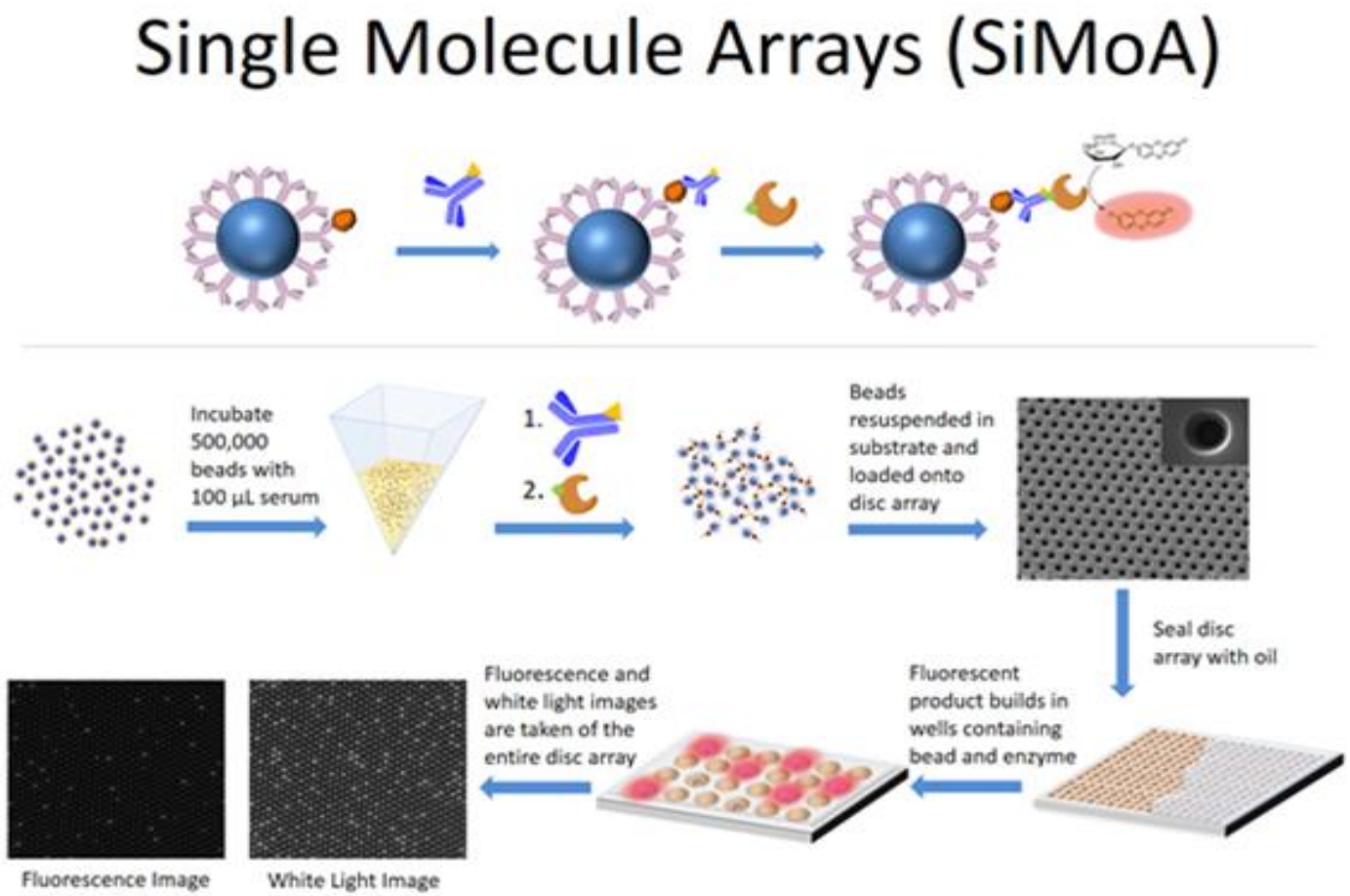
LBA methods for Biomarker evaluation



Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Why SIMOA, who needs it, and what for?

- Highly sensitive measurements in the low pg/ml or high fg/ml range

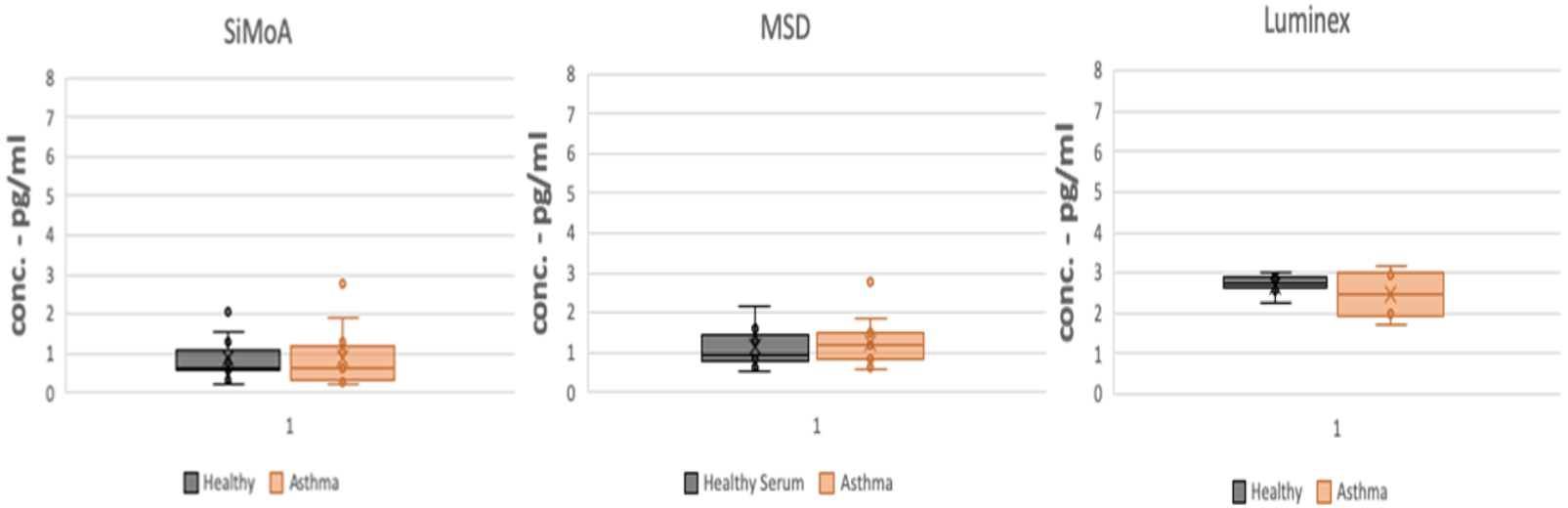
- New very potent drugs are active in this low range and SIMOA is the “only” technology able to measure at such low levels

- Many Biomarkers, particularly in chronic diseases show small changes in this low analytical range (Biomarker Support)

- Alleviates patient burden- key Biomarkers can be measured in Blood instead of CSF (Cerebrospinal Fluid)

Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Respiratory Biomarker: IL-6



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No significant differences for IL-6 were observed between healthy and diseases population, but only SIMOA (!) didn't show any BLQ (below limits of quantitation) values.

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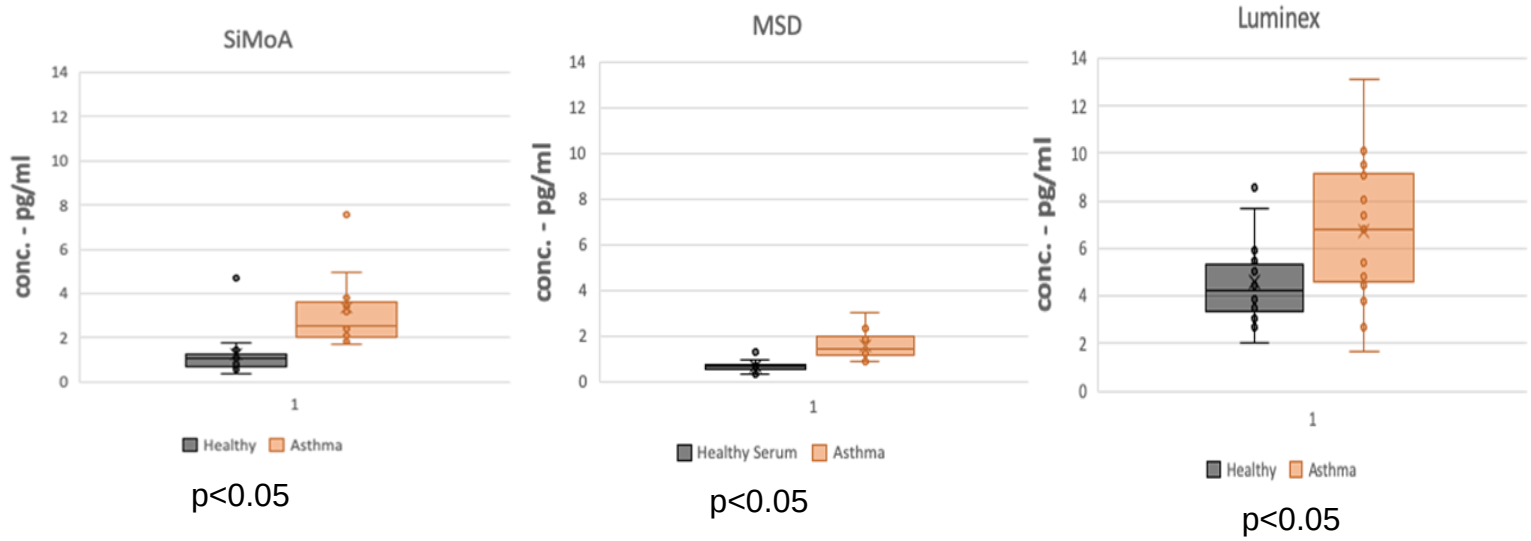
Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Respiratory Biomarkers: TNF-α



TNF-α levels were significantly different ($p<0.05$) in asthmatic patients compared to healthy individuals but only with SIMOA (!) no BLQ values were observed.

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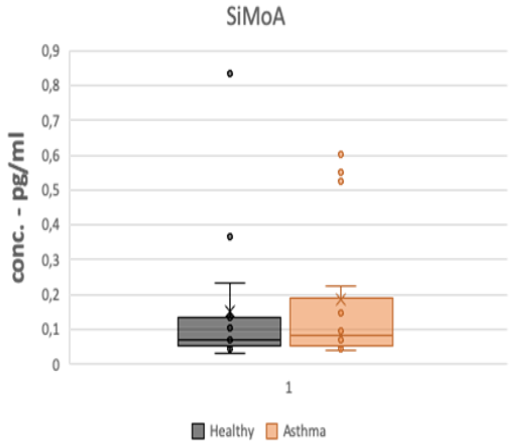
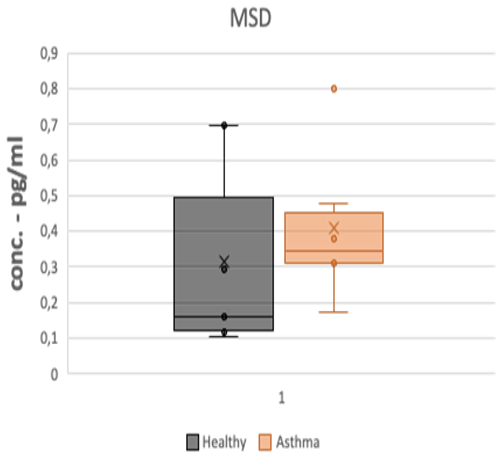
Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Respiratory Biomarkers: IL-13



p<0.1



p>0.1

IL-13 levels showed significantly different values (p<0.1) in asthmatic patients compared to healthy individuals measured with SIMOA (!); no values could be measured by other technologies-MSD values are 100% extrapolated

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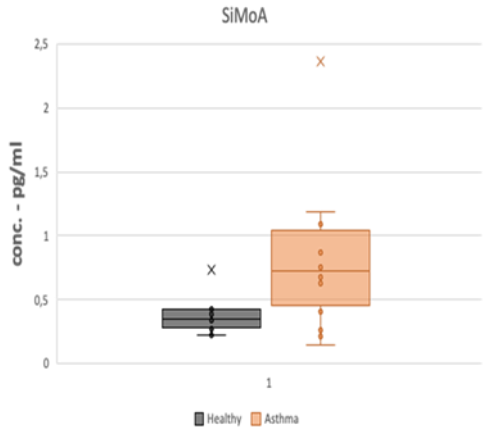
Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Respiratory Biomarkers: IL-5



p<0.05

IL-5 levels showed significantly higher values (p<0.05) in asthmatic patients compared to healthy individuals measured with SIMOA (!); no other technology could measure down to the requested levels.

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Inflammatory Biomarkers: Simoa makes the tiny but significant difference

- Summary SIMOA and Respiratory Biomarkers

- The Key Biomarkers IL-6, TNF-α, IL-13 and IL-5 in Asthma and healthy individuals were analyzed with SIMOA, MSD and Luminex in plasma. This analysis will be extended to a non-invasive sputum analysis in CF patients.

- For all Biomarkers SIMOA demonstrated to be superior compared to MSD or Luminex either because only with SIMOA all samples could be measured (no BLQ values) (IL-6, TNF-α) or because SIMOA was the only technology differentiating healthy from diseased individuals (IL-13 & IL-5)

- Unequivocally the need for SIMOA technology was demonstrated in order to reliably detect Respiratory Biomarkers.

Acknowledgments

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- Heinrich Faust

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