NAVDF 2025

Clinical Investigation and Biomarker Discovery in Early Drug Development for Allergic Dermatitis

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Part 1: Introduction to Biomarkers and Molecular Advancements

Disclosures

- I work for Zoetis Inc.
- Content of this lecture reflects my own personal opinions
- Images created in BioRender.com are noted

Outline

- What is a Biomarker?
- Advancements in Molecular Techniques
- Introduce the “BEST Definitions”
- Clinical Utility and Application
- Q & A

What is a Biomarker?

- Shortened from "**Biological Marker**" (1950's)
- Biomarkers are objective, quantifiable **medical signs** that modern science allows us to measure reproducibly.
- According to the FDA, biomarkers are **essential tools** in personalized medicine and drug development.
- "Any substance, structure, or process that can be **measured** in the body or its products and influence or predict the incidence of outcome or disease" (WHO – joint venture on chemical safety)

1998 NIH-FDA Biomarkers Definitions Working Group:

A **defined characteristic** that is objectively **measured** and evaluated as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.

Contrast “Biomarker” with “Clinical Endpoint”

- Clinical endpoints define a characteristic or variable that reflects how a patient “feels,” functions, or survives.
- Clinical endpoints help researchers evaluate the success of novel treatments and guide regulatory decisions and drug label claims.

A **clinical endpoint** is a measurable outcome used to assess the **effectiveness** of safety and treatment in a clinical trial.

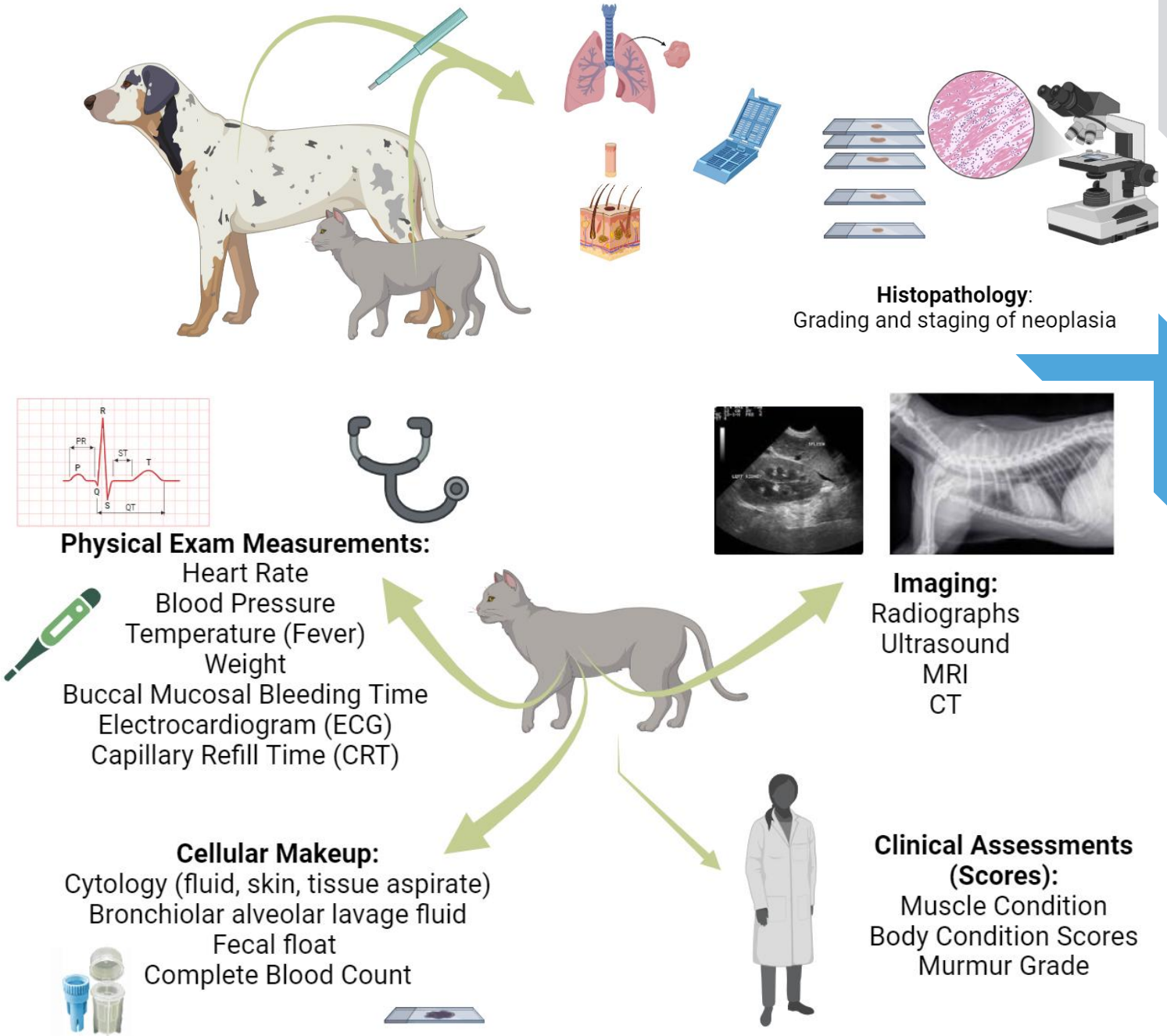
- Some examples include:
  - Overall survival (OS) – time between treatment and death from any cause
  - Progression-Free Survival (PFS) – length of time during and after treatment that a patient lives without the disease worsening
  - Reduction in Clinical Signs (symptoms) – assessing improvements in itching or skin lesions in dermatology cases

Practical Biomarkers

Biomarkers are broadly applicable to the animal health industry, just as they are to human medicine.

Practical biomarkers encompass a wide range of evaluations and include **physiological, radiographic,** and **histological** measurements - performed daily in veterinary clinics around the world.

Biomarkers are a subcategory of medical signs, which are detected and measured through different tests or procedures:



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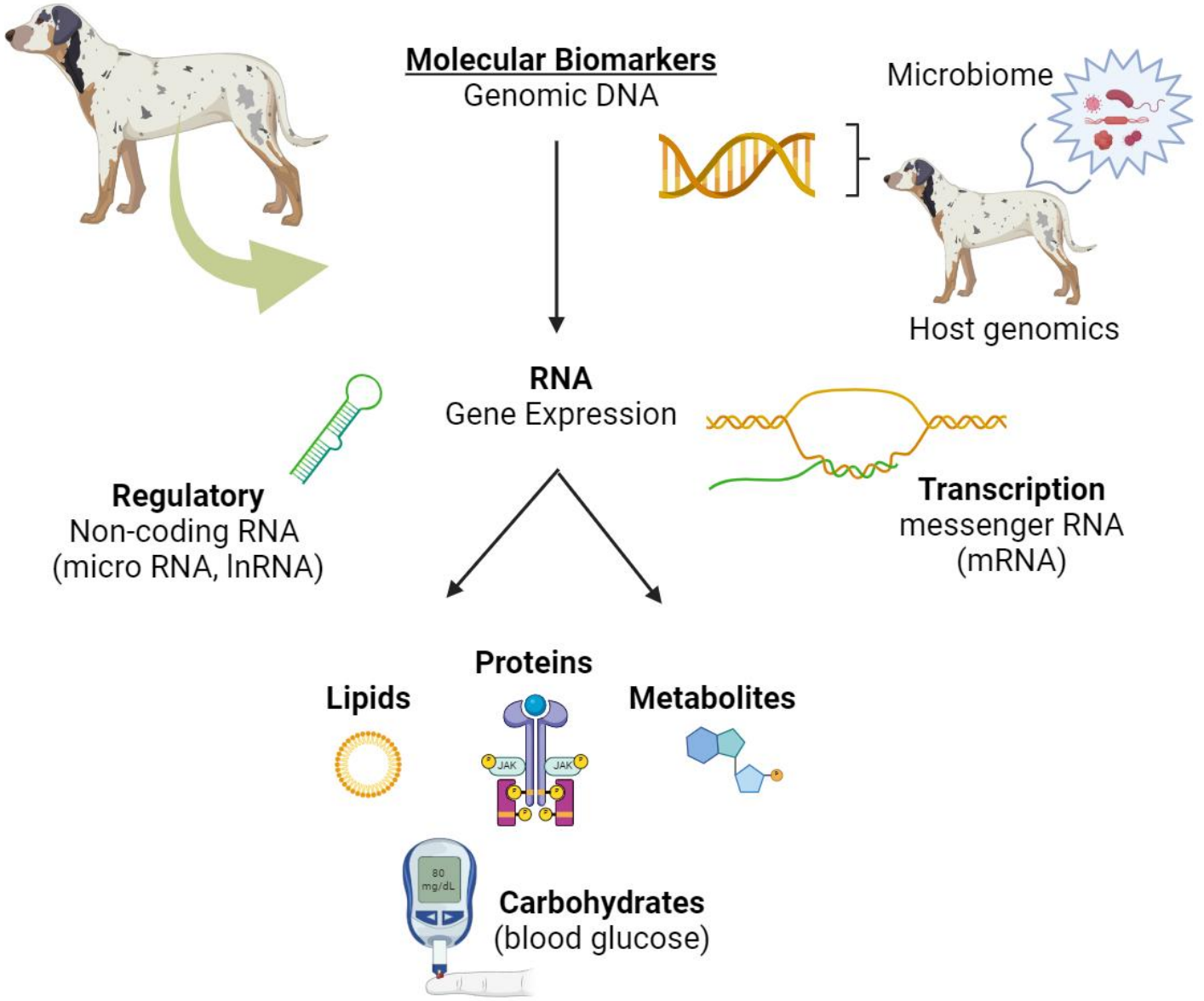
Molecular Biomarkers

The most obvious example of molecular biomarkers in Veterinary Medicine come from standard Clinical Pathology Labs:

- Complete Blood Counts (CBC)
- Comprehensive Chemistry
- Urinalysis
- Serological Titers

With advancements in molecular biology, we can evaluate more than circulating cells or proteins.



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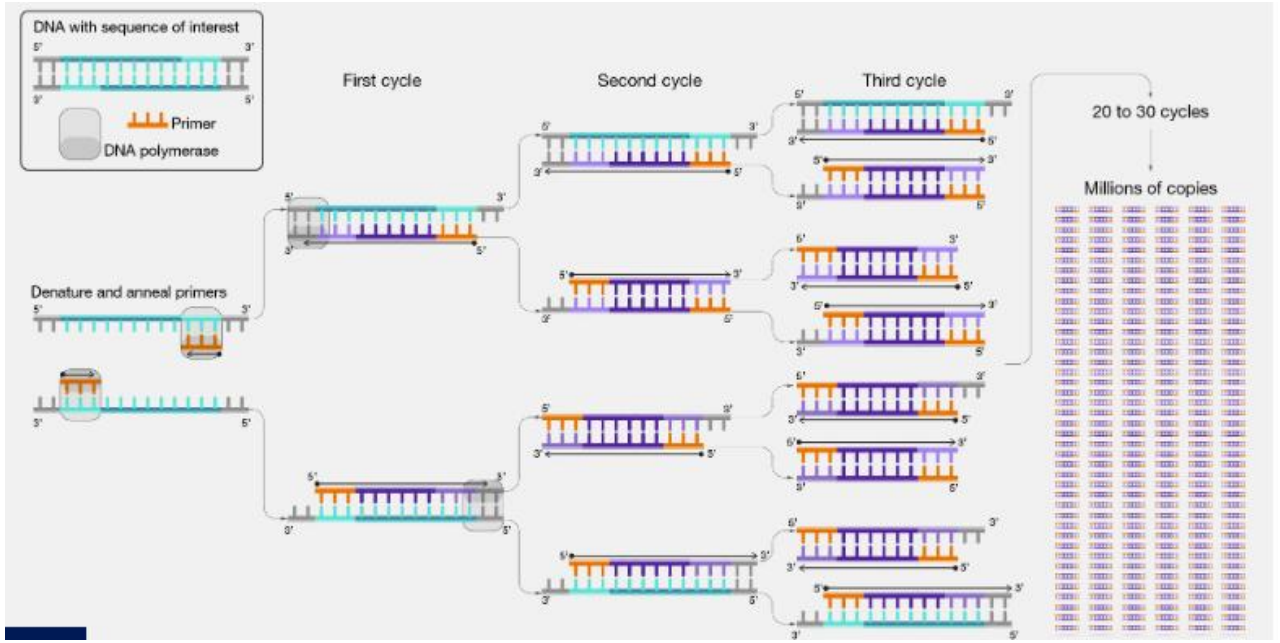
Advancements in Molecular Techniques

Advancements in Molecular Techniques: PCR

Polymerase chain reaction (PCR), is a laboratory technique for rapidly amplifying millions to billions of copies of a **specific segment** of nucleic acid, **DNA or RNA**.

- PCR involves using short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment.

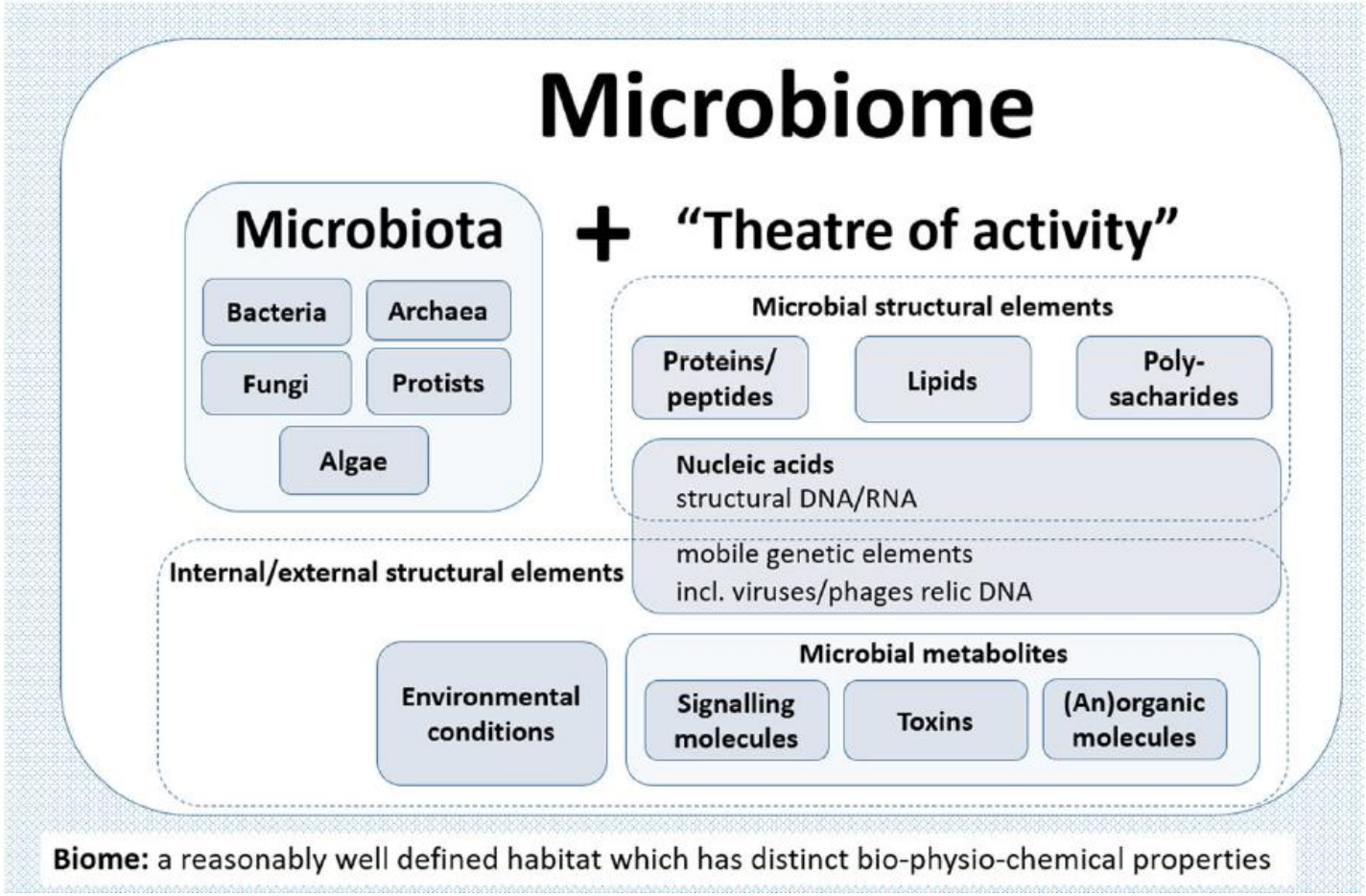


- Viruses with **RNA** as genomic material, first have a step that **converts RNA into DNA** before amplification. This is called reverse transcription PCR (rtPCR).

Talking Glossary of Genetic Terms | NHGRI (National Human Genome Research Institute) www.genome.gov/genetics-glossary

Advancements in Molecular Techniques: Microbiome

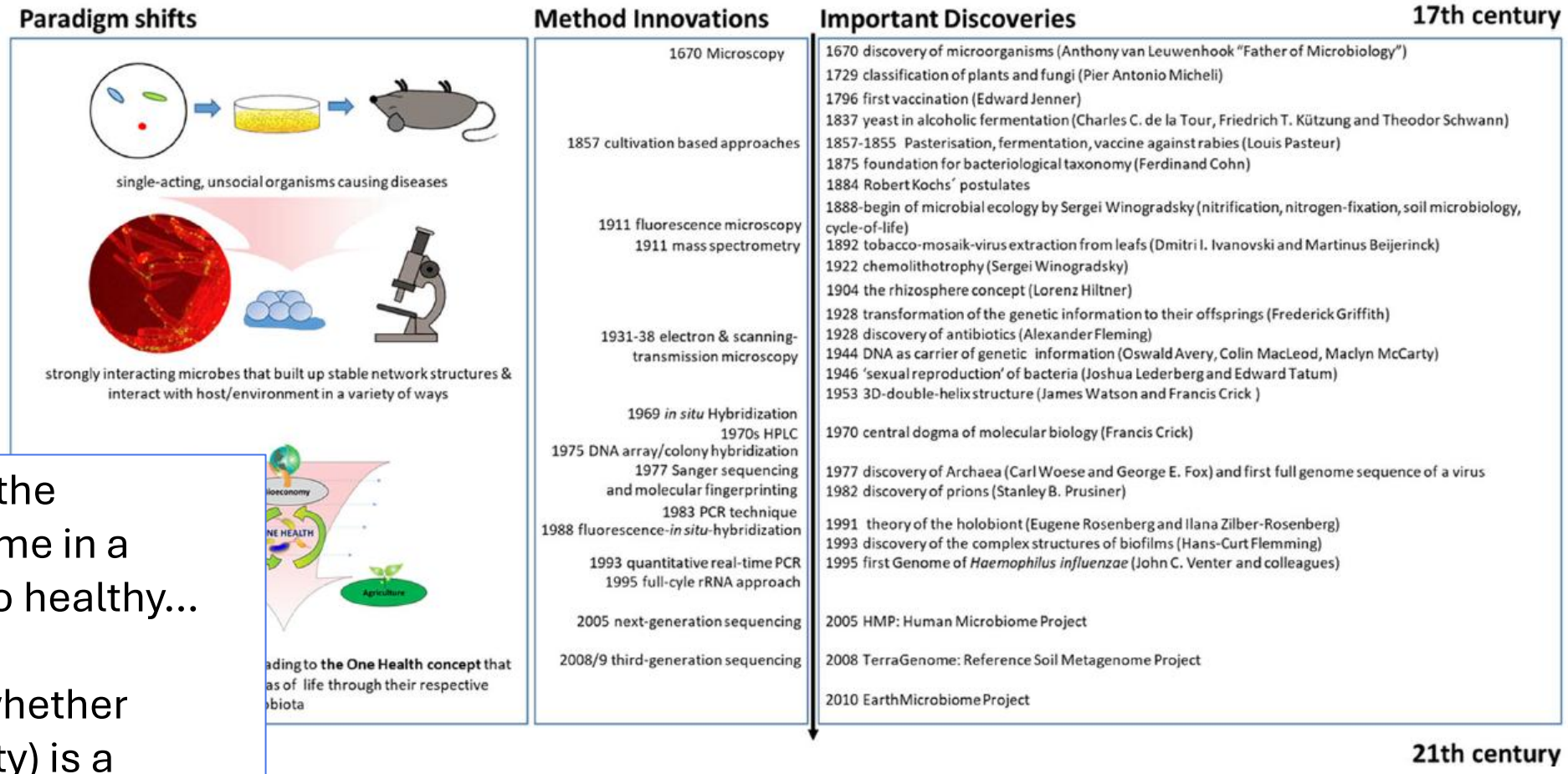
Defined as the characteristic **microbial community** occupying a reasonable well-defined habitat which has distinct physio-chemical properties.



Berg G, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):103. doi:10.1186/s40168-020-00875-0

Advancements in Molecular Techniques: Microbiome

Shift of the paradigm from microbes as unsocial organisms causing diseases to the holistic view of microorganisms



As we begin to understand the differences in the microbiome in a diseased state compared to healthy…

…we begin to understand whether dysbiosis (or loss of diversity) is a contributor or consequence of disease.

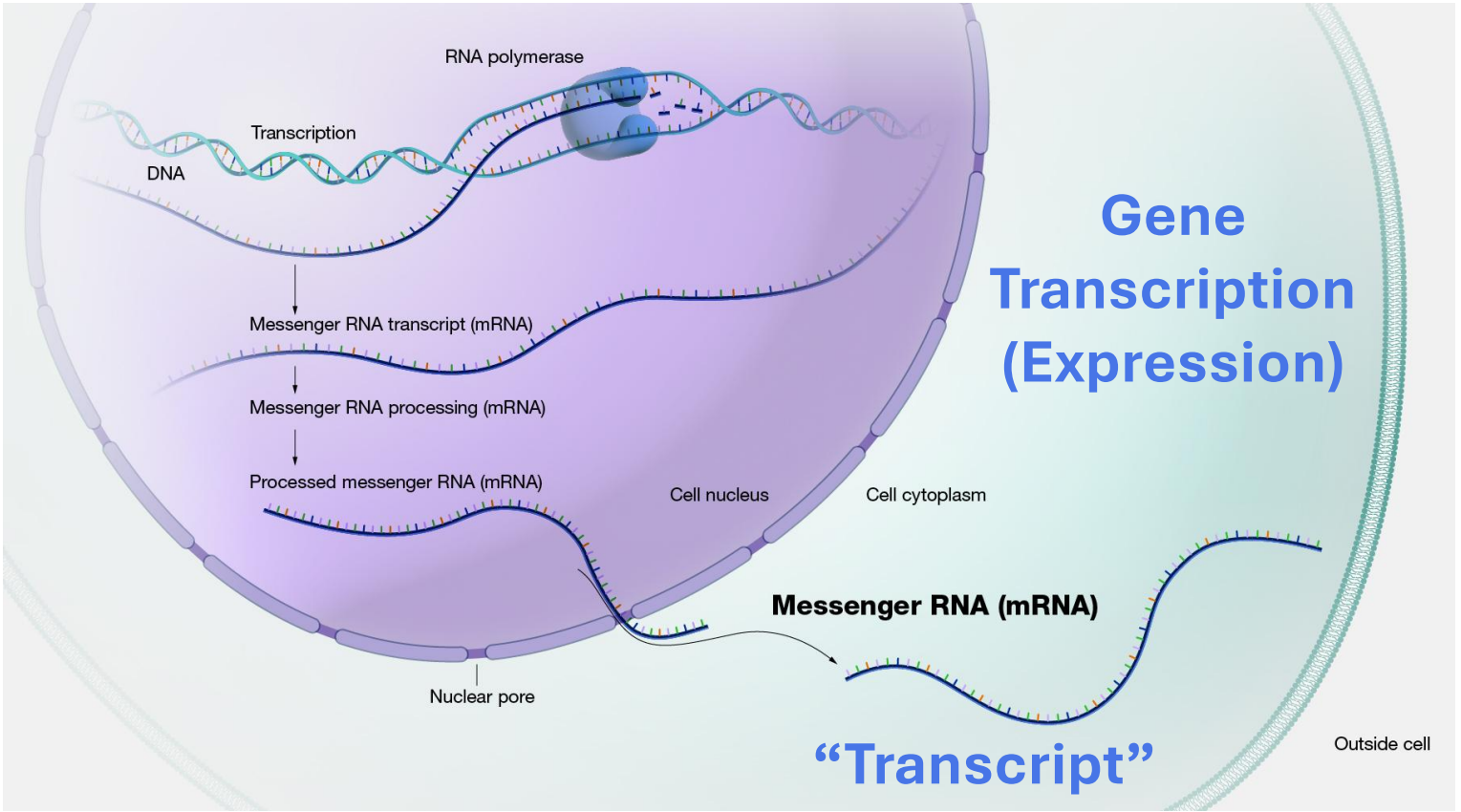
Berg G, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):103. doi:10.1186/s40168-020-00875-0

Advancements in Molecular Techniques: RNA

Functional Genomics- through RNA, genes are actively contributing to cellular processes and responses.

Different types of Ribonucleic Acid (RNA) exist **in cells**, each with a unique function:

- **Messenger RNA (mRNA)**
- Ribosomal RNA (rRNA)
- Transfer RNA (tRNA)
- Regulatory RNAs like microRNA (miRNA), long non-coding RNA (lncRNA), small interfering RNA (siRNA)

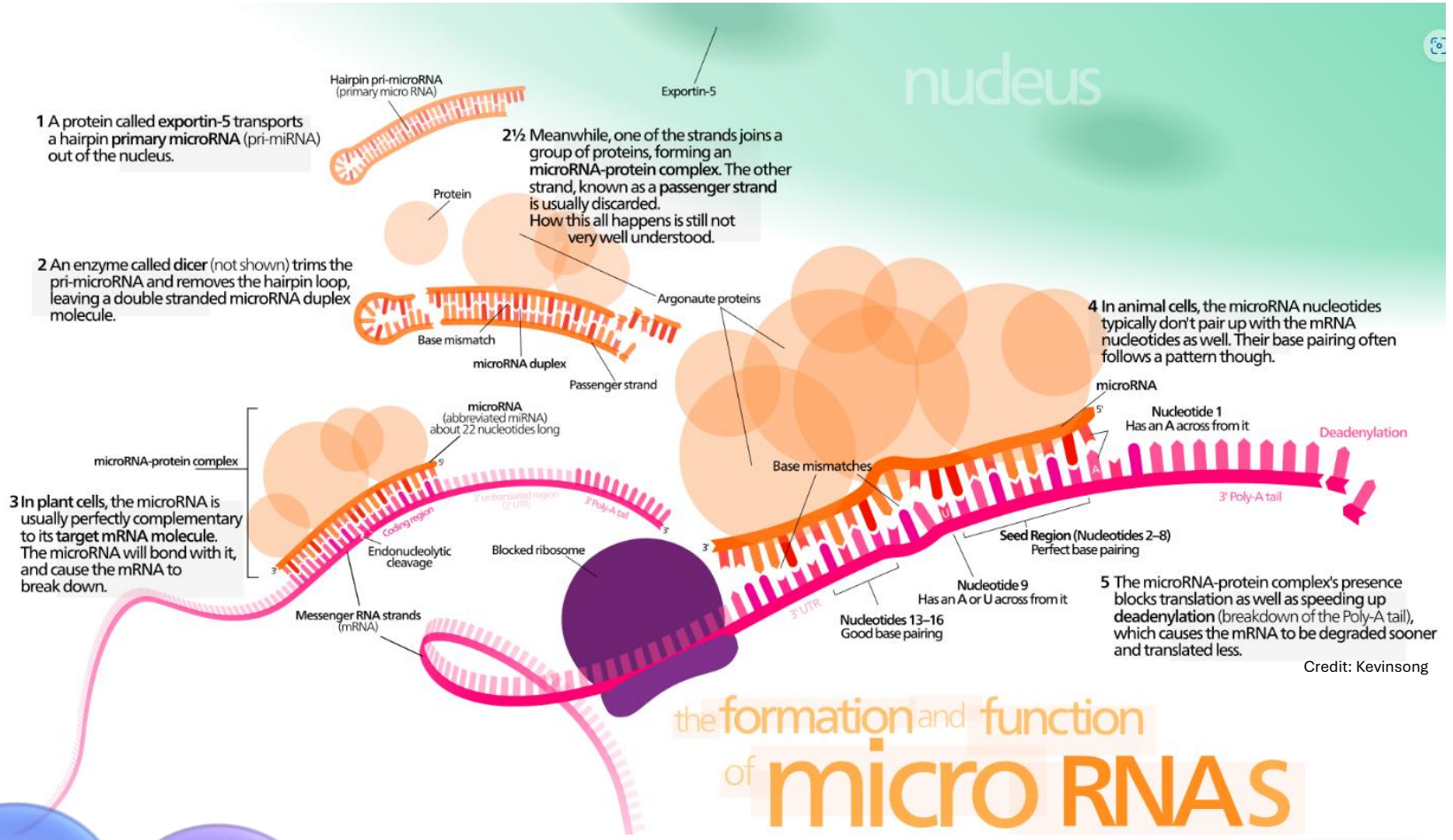


Central Dogma:
DNA → RNA → Protein

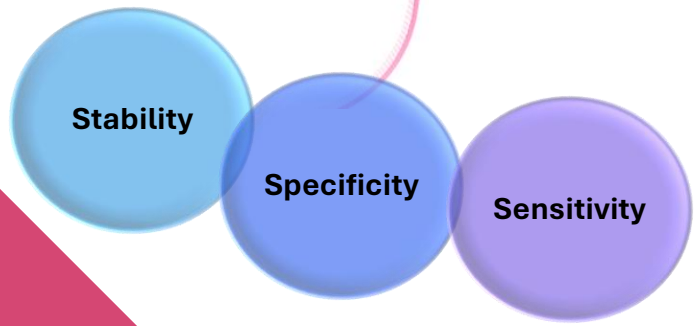
Talking Glossary of Genetic Terms | NHGRI (National Human Genome Research Institute) www.genome.gov/genetics-glossary

Advancements in Molecular Techniques: microRNAs

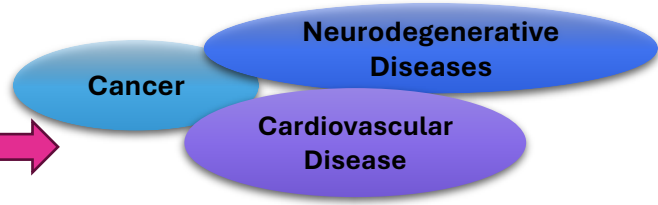
MicroRNAs regulate gene expression through mRNA degradation or translational repression by specific binding to mRNA and targeting transcripts into RISC complexes to either be broken down or simply interfering with the ribosome translation.



Credit: Kevinsong



miRNAs promising biomarkers

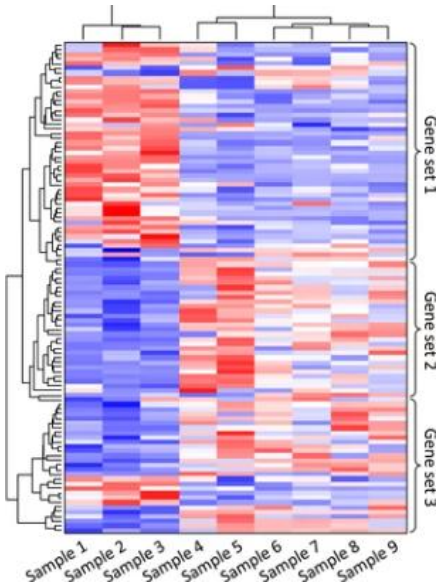


Advancement in Molecular Techniques: Transcriptomics

Functional Genomics & Transcriptomics: both profiles and quantifies the EXPRESSION of genes.
To understand the identity, abundance, structure, and regulation of RNA molecules with relationship to disease phenotype or mechanism.

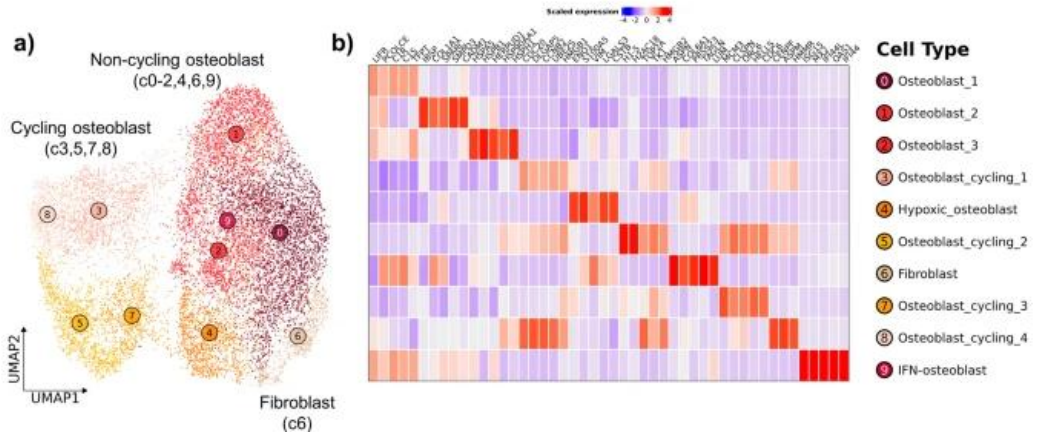
RNA-sequencing (RNA-seq)

- High-throughput sequencing of RNA molecules, providing bulk information about the types and quantities of RNA transcripts present in a **biological sample**. *Gene sets*
- Differential expression analysis enables the **comparison** of gene expression between different conditions (samples). *Heat maps*



Single cell RNA-sequencing (scRNA-seq)

- Gene expression **at the single-cell level** within a sample.
- Spatial transcriptomics enables researchers to **map gene activity** of individual cells maintaining the **spatial context** of the cell populations and allowing for contextual analysis of tissue architecture. *Cell Types & UMAPs*



Ammons, D.T. et al. (2024). Single-cell RNA sequencing reveals the cellular and molecular heterogeneity of treatment-naïve primary osteosarcoma in dogs. Commun Biol, 7, 496.

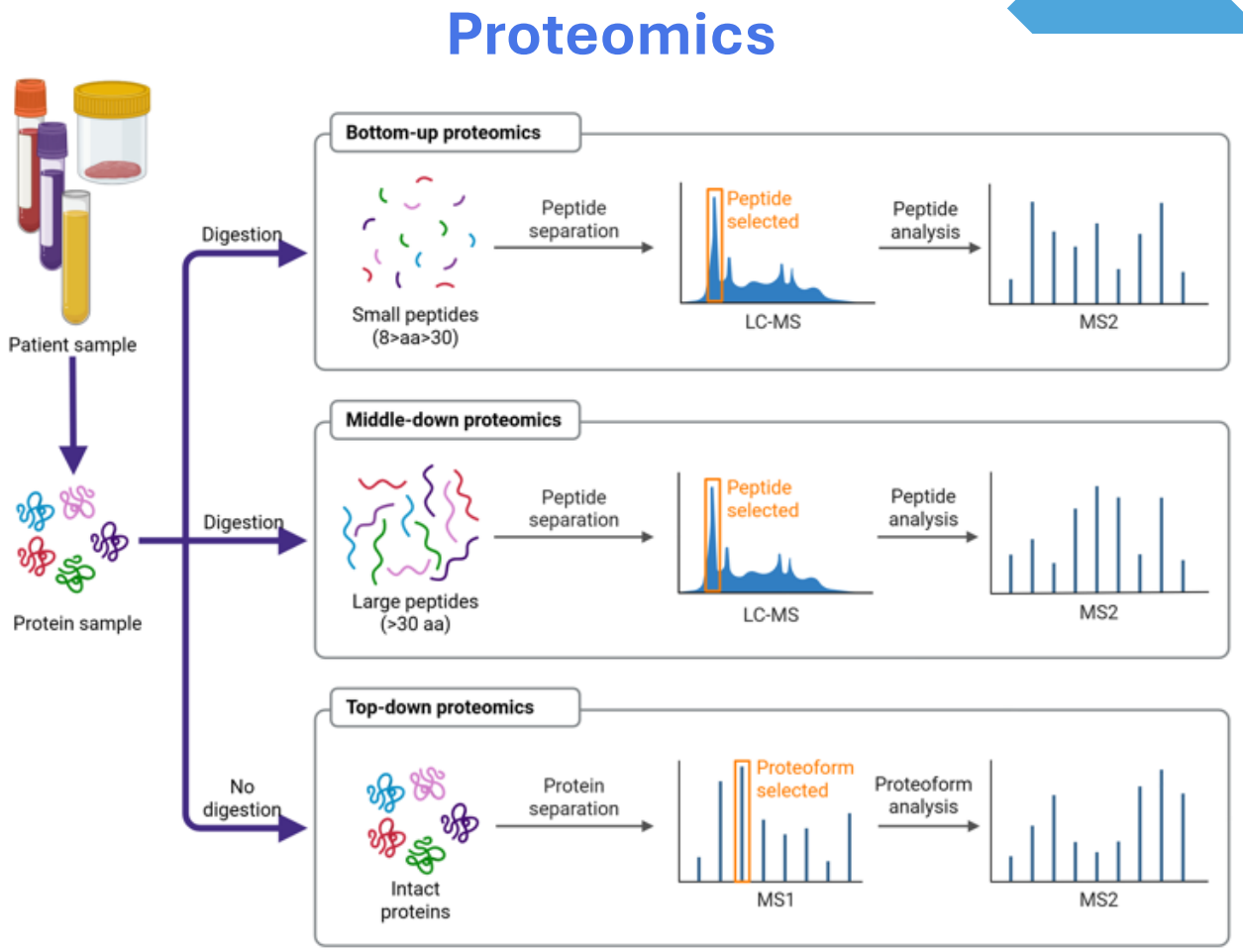
Advancement in Molecular Techniques: Muti-omics

A discipline that combines data from the multiple “omics”

- Proteomics
- Metabolomics
- Lipidomics
- Transcriptomics

Less bias -avoids a targeted look at specific molecules analyte-by-analyte

Scientist gain deeper insights into complex mechanisms and biomarker signatures with high throughput



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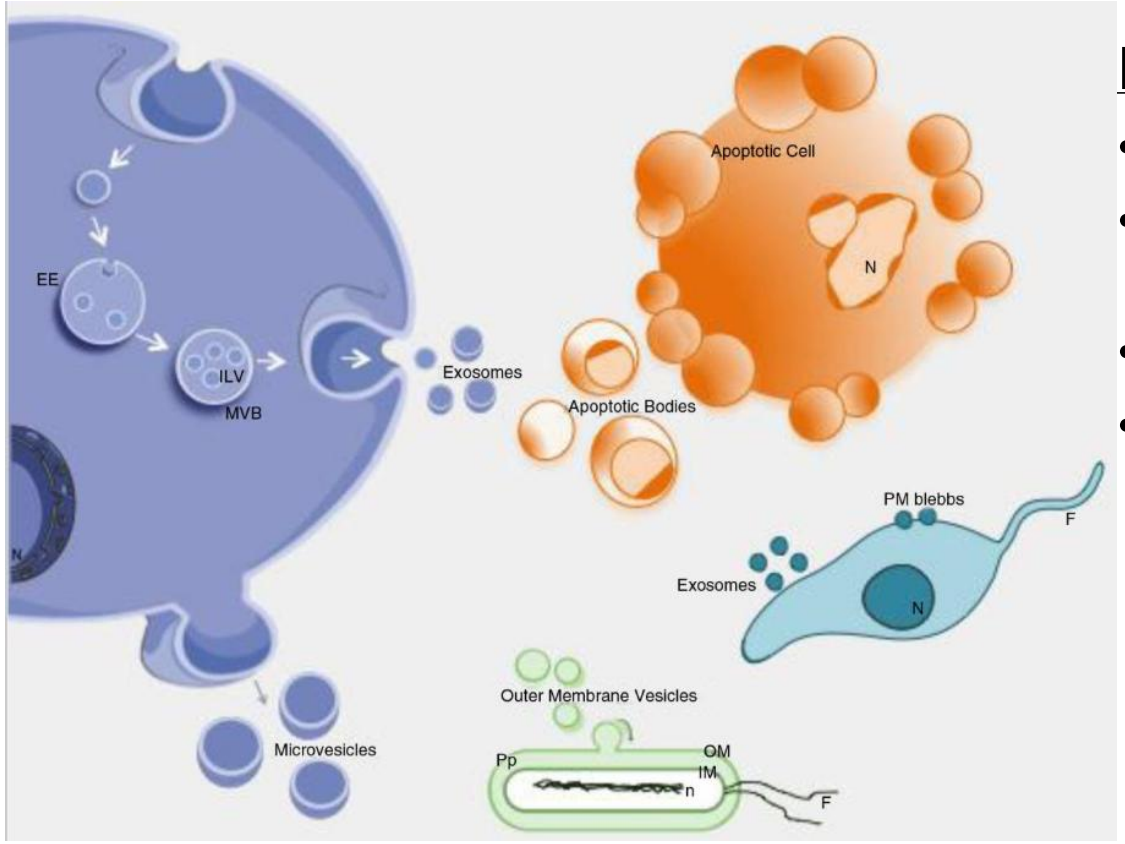
Advancement in Molecular Techniques: Cellular Entities - EVs



Extracellular Vesicles (EVs)

- Lipid bilayer particles typically < 200 nm in size.
- Actively released from cell membrane for intracellular communication, by nature in circulation.
- Types: Apoptotic Bodies, Microvesicles, Exosomes
- Bacteria, Protozoa, Parasites, Enveloped Viruses

Diverse cargo:

- Proteins
- Metabolites
- Lipids
- Nucleic acids (DNA, RNA)

Prime candidates for sampling molecular signatures!

Multi-omics

Non-invasive collection

Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*. 2015;4:27066. Published 2015 May 14. doi:10.3402/jev.v4.27066

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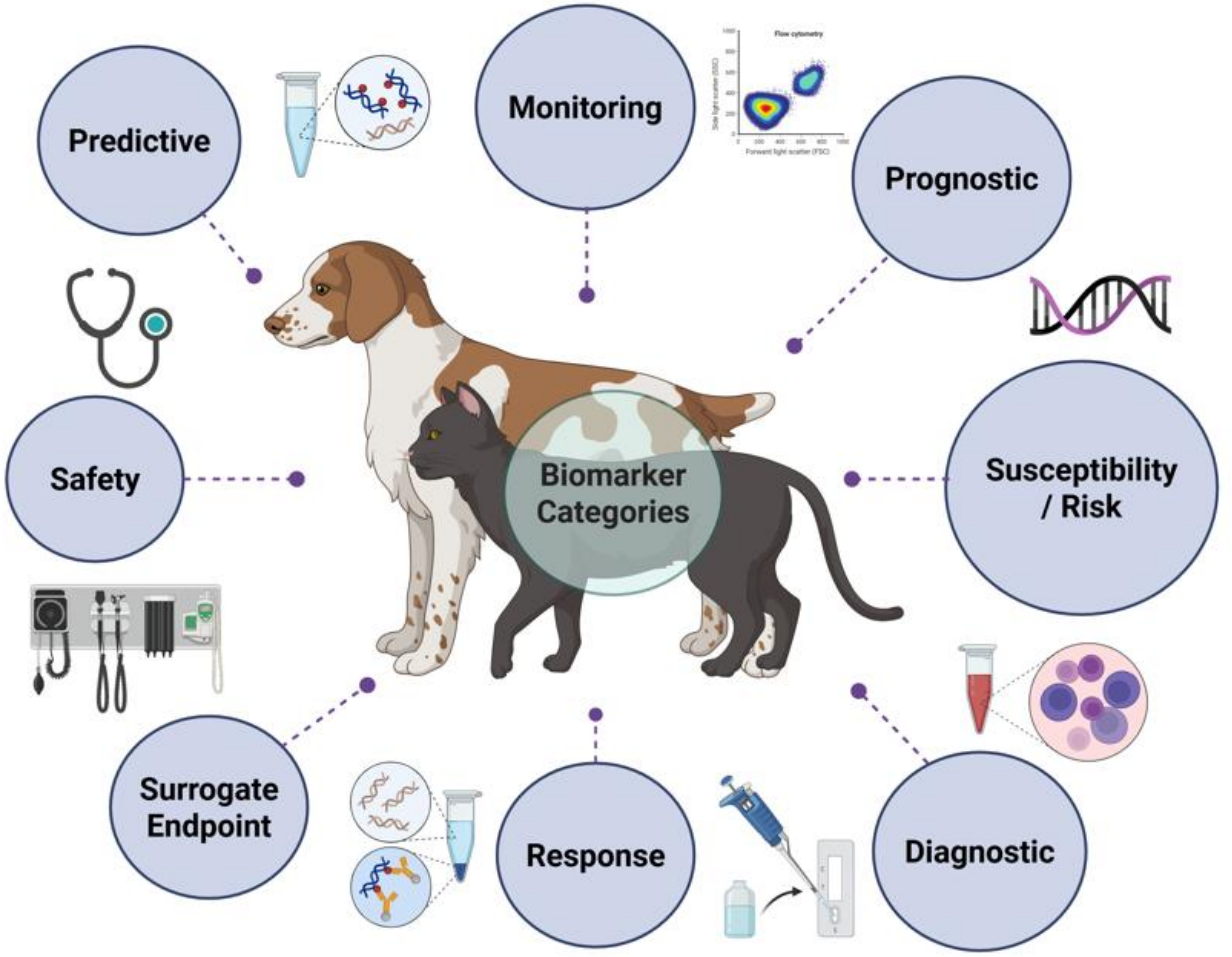
BEST Definitions

Introduction to:
Biomarker, EndpointS, and other Tools (BEST) Definitions

- In 2016, an FDA-NIH Joint Leadership Council published the **B**iomarkers, **E**ndpoint**S**, and other **T**ools (BEST)
- Resource to promote consistent use of biomarker terms and concepts, align expectations, and improve scientific understanding of study endpoints and biomarkers.
- As of 2025, the BEST glossary classifies biomarkers into eight specific categories.
- The BEST glossary is a “living” resource that is periodically updated with additional terms and clarifying information aiming to capture distinctions between biomarkers and clinical assessments.
- Defines each category and provides references and specific examples.
- In practical purposes, the BEST definitions are the application of biomarkers in everyday use and is a shared dictionary between industry, academia, and the US regulatory agencies to advance drug development.



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BEST Resource: https://www.ncbi.nlm.nih.gov/books/NBK326791/ Co-published by National Institutes of Health (US), Bethesda (MD) FDA-NIH Biomarker Working Group.

A **Diagnostic Biomarker** is 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.

Diagnostic biomarkers are used for the critical determination of whether a patient has a particular medical condition; with an accurate diagnosis of a disease or condition a specific treatment may be indicated.

Example:
Detecting “Giardia Cyst Antigen” in a patient’s fecal sample.

Vetscan Giardia Rapid Test

CANINE INFECTIOUS, FECAL

A highly sensitive and specific test for the qualitative detection of giardia cyst antigens in canine feces.

SENSITIVITY (CANINE): 98.1%[1]

SPECIFICITY (CANINE): 99.3%[1]

Diagnostic Biomarker

Monitoring Biomarker

A **Monitoring Biomarker** is a biomarker measured repeatedly for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent.

Evaluated serially over time, a **Monitoring Biomarker** is often used to assess disease progression, including the occurrence of new disease effects, worsening of previously existing abnormalities, or change in disease severity or specific abnormalities.

Response Biomarkers

A **Response Biomarker** is a biomarker used to show that a biological response, potentially beneficial or harmful, has occurred in an individual (or animal) exposed to a medical product or an environmental agent.

A response biomarker can be further divided into **Pharmacodynamic** biomarker and **Surrogate endpoint** biomarker and depends on its specific “context of use”.

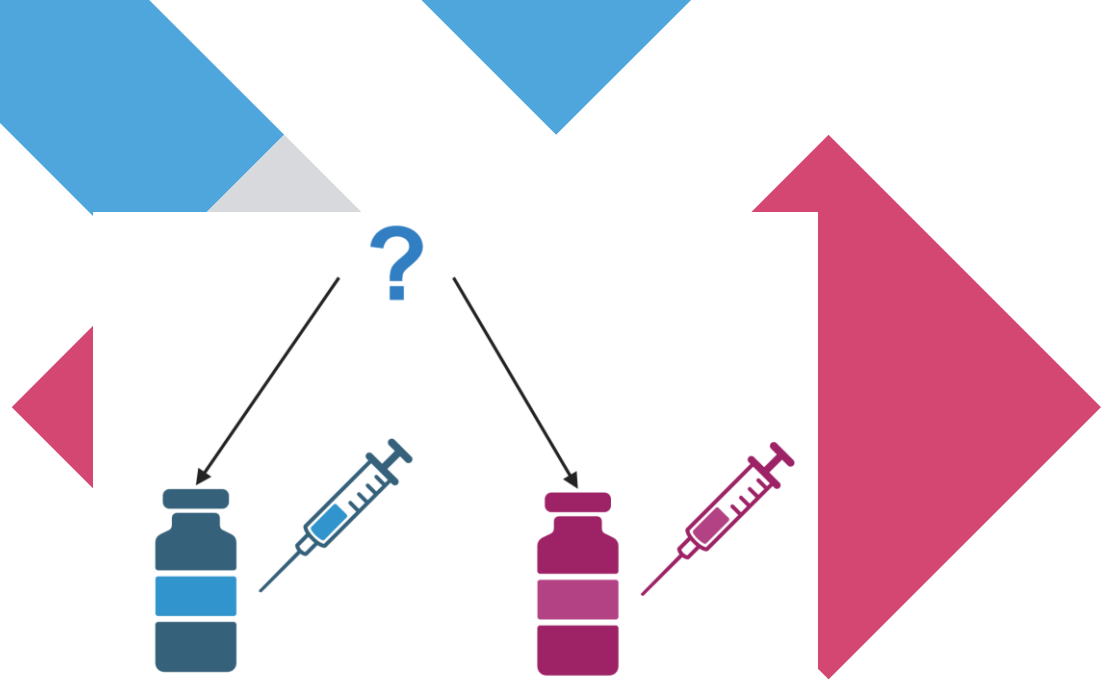
Pharmacodynamic (PD) Biomarkers: a response biomarker that indicates biologic activity of a medical product (i.e.; therapeutic intervention); used to establish proof-of-concept, assist in dose selection, or measure potential harm.

Surrogate Endpoint: a response biomarker used in clinical trials as a substitute for direct measure of how a patient ‘feels’, functions, or survives (but does not measure the clinical benefit of primary interest in and of itself).

Predictive Biomarkers

A **Predictive Biomarker** is 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.

Predictive biomarkers are often used in clinical trials to select patients for participation or to **stratify patients** into biomarker groups (positive or negative) with the primary endpoint being the effect in the biomarker positive group.



Personalized medicine, what is the best treatment option based on objective measurements....

Prognostic Biomarkers

A **Prognostic Biomarker** is
a biomarker used to identify likelihood of a clinical event (such as death), disease recurrence or progression in patients who have the disease or medical condition of interest.

In the clinical context, prognostic biomarkers are measured at a **defined baseline**.

The BEST glossary makes a distinction between prognostic biomarkers & susceptibility/risk biomarkers…

Susceptibility Biomarkers – individual without clinically apparent disease

Prognostic Biomarkers – patient has the disease… using a biomarker to inform "prognosis"

Susceptibility / Risk Biomarker

A **Susceptibility/Risk Biomarker** is a biomarker that indicates the **potential for** developing a disease or medical condition in an individual who **does not currently have** clinically apparent disease or the medical condition.

..contrasts with Prognostic Biomarkers, which indicate an increased likelihood of a specific clinical event in an individual already diagnosed with a disease or medical condition

...contrasts with Diagnostic Biomarkers, which confirm whether a disease is present

Reasonably Likely Surrogate Endpoint

A **Reasonably Likely Surrogate Endpoint** is an endpoint supported by **strong mechanistic** and/or epidemiologic rationale such that:

1. an effect on the surrogate endpoint is expected to correlate with an endpoint intended to assess clinical benefit in clinical trials

2. but without sufficient clinical data to show that it is a validated surrogate endpoint

May be used to accelerate approval for drugs…

… often with post marketing confirmatory trials to verify and describe the anticipated effect on the irreversible morbidity, mortality, or other clinical benefit

A **Safety Biomarker** is
a biomarker measured before and after an exposure to a medical product or an environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect.

Common to all safety biomarkers is the ability to detect or predict these adverse drug or exposure effects.

Safety Biomarker

Introduction to:
Biomarker, EndpointS, and other Tools (BEST) Definitions

BEST Definitions from FDA / Christopher Leptak	
Biomarker class - from a drug development perspective	
Susceptibility - Risk	Indicates potential for developing disease before it is clinically apparent.
Diagnostic	Confirms presence of disease or condition or identifies patient subsets.
Monitoring	A biomarker measured repeatedly (i.e.; serially) for assessing the disease status, including degree or extent (**severity**), or for evidence of exposure to (or effect of) a medical product or an environmental agent.
Prognostic	Identifies likelihood of a clinical event, disease recurrence or progression in the absence of a therapeutic intervention.
Predictive	Identifies patients who are more likely to experience a favorable or unfavorable effect from a specific treatment or exposure. (Establishing that a biomarker is predictive for an intervention's effect generally requires a comparison of the intervention to a control treatment in individuals with and without the biomarker, usually in randomized trials.)
Response (Pharmacodynamic or Surrogate Endpoint)	**Pharmacodynamic Biomarker**: a response biomarker that indicates biologic activity of a medical product (i.e.; therapeutic intervention); used to establish proof-of-concept, assist in dose selection, or measure potential harm. **Surrogate Endpoint**: a response biomarker used in clinical trials as a substitute for direct measure of how a patient 'feels', functions, or survives (but does not measure the clinical benefit of primary interest in and of itself).
Safety	Indicates the likelihood, presence or extent of toxicity to a therapeutic intervention when measured before or after the intervention.

Available from: https://www.ncbi.nlm.nih.gov/books/NBK326791/ Co-published by National Institutes of Health (US), Bethesda (MD) FDA-NIH Biomarker Working Group.

Clinical Utility

Clinical Utility & Application

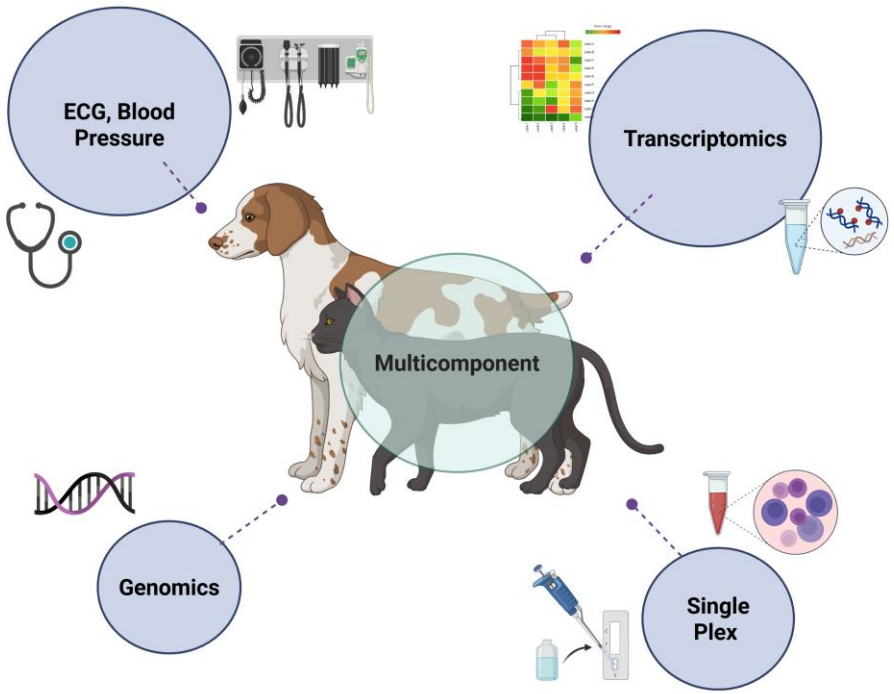
Contents of a Biomarker Description:

- Added to the BEST glossary in 2020 to succinctly summarize key aspects necessary for universal “biomarker description”

1. The **biomarker identity**:
   a. name of the specific analyte, anatomic feature, or physiological characteristic as well as unique identifiers or commonly used acronyms.
   b. The specific source (e.g., urine, liver, biopsy, etc.).
   c. The type of biomarker (e.g., physiological, molecular, histological, etc.).
2. The **biologic plausibility:** summary of the biological, physiological, or pathological pathway for the association of biomarker with disease or condition. “Contextual linkage between biomarker and intended use”
3. **Measurement method:** used to measure, image, or otherwise quantify the biomarker with details to interpret the results (e.g., units)

Biomarker Signatures & Multicomponent Biomarkers (MCB)

There are many situations where multiple single biomarkers may be relevant to a particular context of use:



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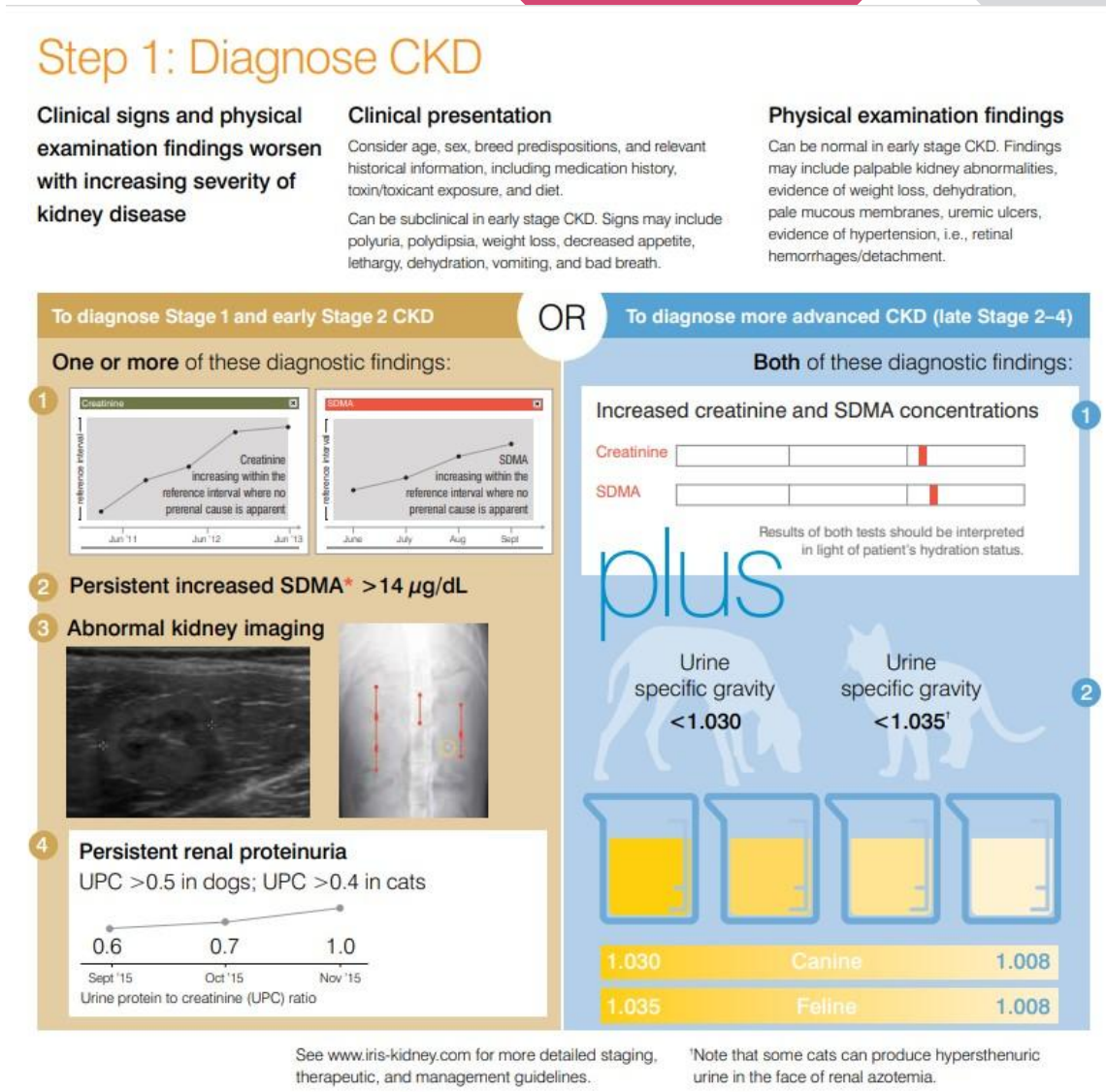
Using a combination or defined set of two or more individual biomarkers whose values, when considered together in a specified way provide an indicator of normal biological processes pathogenic processes or biological responses to an exposure or intervention including therapeutic interventions.

Biomarker Signatures & Multicomponent Biomarkers

2019 Antech Diagnostics release RenalTech® - a predictive diagnostic tool indicating a cat's likelihood to develop CKD

- RenalTech® uses common blood and urine tests that you already run routinely on your feline patients, combined with the pet's age.
- The artificial intelligence that powers RenalTech® uses results from two diagnostic testing events: one current diagnostic event can be paired with another set of results captured within the last 24 months.



IRIS_Pocket_Guide_to_CKD_2023

Clinical Utility & Application

- In human health, there are regulatory processes in place for qualifying and validating biomarkers.
- Additionally, in human health biomarker-driven clinical trials are now mainstream, **with nearly half of oncology studies and 10-20 % of all trials incorporating biomarkers.**
- There is set regulatory path for biomarkers in a clinical trial application.
- There is no regulatory process to approval for animal health biomarkers.
  - **In Veterinary Medicine, we rely on the Key Opinion Leaders, body of literature, as well as commercial organizations to drive the clinical utility and acceptance of biomarkers.**

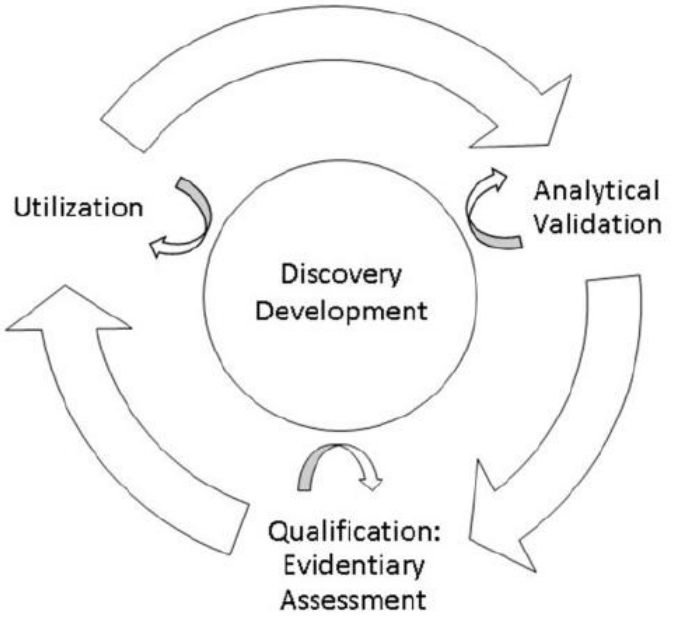


Figure 1. Steps in the evaluation framework for biomarkers. Adapted from: Institute of Medicine. *Evaluation of biomarkers and surrogate endpoints in chronic disease.* Summary. Washington, D.C.: National Academies Press, 2010.

Clinical Investigation and Biomarker Discovery in Early Drug Development for Allergic Dermatitis Part 2: Biomarkers and Translational Biology in Allergic Dermatitis
Tuesday, Apr 29, 2025
2:00 PM - 2:50 PM
Windermere Ballroom W, Convention Level

Thank you

Nikki M. Thellman DVM, PhD

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